

# THE CHEMISTRY OF LEATHER MANUFACTURE

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## GENERAL INTRODUCTION

### American Chemical Society Series • of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's *Handbuch der Organischen Chemie*, Richter's *Lexikon*, Ostwald's *Lehrbuch der Allgemeinen Chemie*, Abegg's and Gmelin-Kraut's *Handbuch der Anorganischen Chemie* and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coördinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of coöperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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## Preface to First Edition.

The chemistry of leather manufacture is progressing more rapidly now than at any previous time. Much of the earlier work failed to recognize the existence of important variable factors and has been rendered obsolete by recent investigations carried out under more highly refined conditions. In preparing this monograph, it was found necessary, for the purpose of correlating existing data, to conduct many special investigations and these are being reported here for the first time. Advance information on investigations under way in other laboratories has been obtained, wherever possible, so that the presentation might be made reasonably complete to the close of the year 1922.

The literature pertaining to leather manufacture is so vast and the views expressed so numerous and divergent as to make an impersonal compilation of all published papers encyclopedic in size, bewildering to the average reader, and an undertaking of questionable value. In order to fulfill the first purpose of this series of monographs, namely, to present the knowledge available in a readable form, intelligible to those whose activities may be along a wholly different line, the author has felt compelled to present the subject from his own viewpoint, making no attempt to discuss views which, in his opinion, fail to contribute anything to the development of leather chemistry. In so doing, the author is fully aware that there are others who do not share his opinions of the relative merits of various views, but he can only admit his inability to present adequately views which appear to him unsound. But, in a field so vast, there is ample room for as many volumes as there may be sides to the question worthy of presentation and it is in the preparation of additional volumes that criticism of this attitude may find its best expression.

A considerable amount of space has been devoted to the histology of skin and to the physical chemistry of the proteins because of their fundamental bearing on the chemistry of leather manufacture. Descriptions of analytic methods and practical details of leather manufacture have been given only where they seemed necessary to make the subject clearer to chemists unfamiliar with tannery routine.

Many of the ideas presented in this book were gained during a period of intimate association with Professor H. R. Procter, of the

University of Leeds, England, who is affectionately known throughout the world as the "father of leather chemistry" and whose books on leather manufacture have been the standard for the past thirty-five years.

In the preparation of sections of photomicrographs, valuable assistance was rendered by Mr. Guido Daub, whose painstaking efforts are largely responsible for the success of this phase of the work. The sections and specimens of human skin were procured from Professor T. H. Bast, of the University of Wisconsin. Professor Arthur W. Thomas, of Columbia University, supplied the skins of guinea pigs and albino rats fixed in Erlicki's fluid. Leathers from the hides of the hippopotamus, walrus, and camel were furnished by Professor Douglas McCandlish, of the University of Leeds. Most of the remaining specimens were provided by the firm of A. F. Gallun & Sons Company, in whose laboratories the work was done. The interesting photographs illustrating the drying of gelatin blocks were furnished by Dr. S. E. Sheppard, of the Eastman Kodak Company.

Grateful acknowledgment is made of the generous criticisms and suggestions given by Mrs. Marion Hines Loeb, of the University of Chicago, on the general histology of skin; by Dr. Jacques Loeb, of the Rockefeller Institute, on the physical chemistry of the proteins; and by Professor A. W. Thomas, Mr. Frank L. Seymour-Jones, and Miss Margaret W. Kelly, of Columbia University, on many important points throughout the book.

The author is most deeply indebted to the late Arthur H. Gallun, whose devotion to the cause of leather chemistry has made available a large portion of the data presented in this book.

J. A. W.

Milwaukee, Wisconsin,  
March 12, 1923.

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# THE CHEMISTRY OF LEATHER MANUFACTURE

## Chapter I.

### Introduction.

Leather chemistry is one of the most fascinating branches of industrial chemistry and also one of the most complex, dealing, as it does, with reactions between those poorly defined groups of substances, usually colloidal, whose compositions are still matters of speculation. The raw skin is composed largely of various kinds of protein matter and is complicated by a structure which varies considerably in different animals and even in different parts of the same skin. Conversion into leather involves the removal of some of these proteins by the action of alkalies, enzymes, or bacteria, and the interaction of the remainder with tanning materials, oils, soaps, emulsions, mordants, dyestuffs, gums, resins, and other complex materials. During these reactions the structure of the skin must be carefully preserved, or improved, and highly developed technic is required to impart to the resulting leather certain necessary, but almost indefinable, properties, many of which it is an art even to appreciate fully. When one considers the vast amount of energy expended by organic chemists upon the materials involved in making leather and the uncertainty of our knowledge concerning the individual substances, the complexity of the whole problem becomes more apparent.

Leather manufacture as an art probably antedates chemistry as a science. Well preserved specimens of leather from ancient Egypt bear testimony to the high state of development of the art over three thousand years ago. Its origin presumably dates back to the time when man first began to kill animals for food. The skins, not being palatable, were very likely discarded at first, but the value of dried skins for clothing or protective covering, could hardly remain long undiscovered. Dried skins are hard and stiff, but would become considerably softer and more pliable after being bent and worked during

use, and it was probably noticed very early that this softening action is more pronounced if the skins are worked while being dried, especially in the presence of fats, such as would naturally cling to the skins of animals crudely flayed. In rainy seasons, when the skins could not be dried rapidly, putrefaction of the epidermal cells would cause the hair to slip and reveal the advantages of un haired skins for certain purposes. The tanning and coloring actions of leaves, barks, and woods were probably also accidental discoveries of a prehistoric age. In fact, many of the tannery operations in use today are of ancient origin.

Secrecy and lack of accurate records make it difficult to follow the evolution of the art, especially in the matter of details essential to the production of the finer qualities of leather. But developments have not all been made by rule-of-thumb methods, as has often been supposed. The great success of a certain class of tanners, for example, has been due to the development of a science of leather manufacture, as distinct from the art, based upon a belief in the constancy of natural laws and involving the organization and classification of countless facts gained by experience or handed down from previous generations.

Modern leather chemistry has grown out of the union of two great sciences, chemistry and the science of the tanner, which has developed through the ages, with little aid from chemistry, and which has contributed a great storehouse of more or less correlated facts not to be found in the chemical literature. Training in leather chemistry is very difficult and time consuming. The chemist who would enter this field must first become as thoroughly versed in the fundamentals of pure chemistry as possible. Upon leaving the university, it is desirable to enter a tannery as one not claiming to know anything about the science of making leather, but with a determination to master the science of the tanner, even though it may require years of study and labor. To be a successful leather chemist requires being both chemist and tanner.

Disillusionment has been common among chemists entering this industry, as the result of the unexpected intricacy of the application of chemistry to leather manufacture, of insufficient training, of false notions of superiority over artisans who had devoted their lives to the industry, or of failure to appreciate that the tanner's own science is usually far more reliable than the chemistry of a beginner in the industry.

The leather industry desires answers to a great many questions, but these questions are not clearly defined. In order to get the answer

to a general question, it is necessary to separate it into the many specific questions it contains and then to put each specific question to Nature separately in the form of an experiment. Chemists are trained to plan and execute experiments and to interpret the results, but they must also be able to recognize and to analyze the question whose answer is sought. It is in this analysis that the chemist requires the training and art of the tanner. Without this training, he is unable to define the specific problems upon whose solutions the advancement of the industry depends.

In order to make substantial progress, the chemist just entering the industry must first, as a rule, devote himself completely to a study and explanation of the mechanism of each step of a process already in successful operation and without in any way interfering with the operation of that process. At the same time, it is desirable to make periodic analyses of the leather at different stages and of the liquors used to treat the skins. These should be charted at frequent intervals and any definite trends with time noted. When these have been plotted for a sufficiently long time, it can be observed which variables being plotted move in sympathy with variations in properties of the finished leather. This practice has proved extremely valuable in establishing tannery controls. In time, the chemist is able to develop satisfactory explanations of the mechanism of existing processes and these are of incalculable value in suggesting practical experiments leading to the elimination of unnecessary operations and to the improvement and further development of others.

One of the greatest disappointments awaiting the chemist who attempts pioneering work in a tannery is to find that whatever change he institutes in a given operation results in lowering the quality of the finished leather. For a time, he may come to regard the tanners who developed the process as supermen. But the explanation lies in the fact that the details of all of the many operations involved in tanning are so interrelated that any change in one operation upsets a balance and requires an adjustment in the other operations, without which the leather will be spoiled. The tanners who developed the process learned how to juggle all of the operations until a satisfactory piece of leather was obtained and then they held rigidly to the process. Each operation was then adjusted to the point where the best result was obtained. After that, the tanner never dared deviate from the established practice and, by the law of chance, anyone else could not hope to change only one factor and get a better result. Actually chemists have often found that one operation was merely neutralizing

the bad effects of another. The elimination of either alone would be fatal to the process. But, when all operations were understood, the two unnecessary operations could be dropped together with advantage.

The burdens placed upon the chemist in the tannery are so great that he needs all the assistance he can get from the chemists doing research in pure chemistry and general fundamentals in the university. In Europe, profitable coöperation between the university and the leather industry was established many years ago. While America was slow to make a start in this direction, it is rapidly assuming a leadership. In 1917, the late Arthur H. Gallun established a fund for research in the fundamentals of leather chemistry, under the direction of Professor Arthur W. Thomas, of Columbia University, with the proviso that all results be published freely for the benefit of the industry as a whole. In the first decade, Dr. Thomas and his collaborators have published more than fifty papers describing work of inestimable value to the industry. In 1920, the Tanners' Council established a leather research laboratory at the University of Cincinnati, under the direction of Professor George D. McLaughlin, who, with his collaborators, has already contributed much valuable information on the histology of skin, curing, soaking, unhairing, and bacteriology. The contributions from both of these institutions are described in detail in the following chapters.

During the past five years, there has also been a decided increase in number of scientific publications emanating from tannery laboratories, some of which are now as well equipped for fundamental research in chemistry as many university research laboratories. During the same period, there has been a widespread increase in appreciation of the advancement of leather chemistry, affecting the processes of the great majority of American tanneries.

It is hoped that the following pages will give chemists in many fields a better understanding of the problems of the leather industry and of the opportunities for coöperative research, and also give the industry itself a clearer appreciation of the possibilities for still further extending the application of pure chemistry to leather manufacture.

## Chapter 2.

### Histology of Skin.

Animal skin is the basis of leather and some knowledge of its intricate structure and complex chemical composition is essential to an appreciation of the extremely complicated reactions involved in making leather. Insufficient information concerning the finer structures of many types of skin so frequently proved the stumbling block in the way of investigations of the mechanism of tannery processes that leather chemists who were impatient to forge ahead found it necessary to become histologists and to make their own investigations of the microstructure of skin. Considerable progress had already been made in the histology of human skin and this work proved to be an invaluable guide to the student of the histology of the skins of lower animals, because most skins possess a common basic structure, but the several different types of skin exhibit such marked differences in details of structure of vital importance in leather manufacture that reasoning only from the histology of one or two specimens of human skin often led to erroneous conclusions. In order to apply histology intelligently to leather manufacture, it was found necessary to make separate studies of every type of skin encountered.

Of the earlier work on the histology of skins used for making leather, that of the late Alfred Seymour-Jones<sup>9</sup> was the most notable and it served as a great stimulus. Less widely known is the work of Henri Boulanger,<sup>1, 2</sup> who published some very fine photomicrographs of skin and leather in 1908. Among the more important contributions which have appeared since the first edition of this book are those of Küntzel,<sup>5</sup> Turley,<sup>10</sup> and McLaughlin and O'Flaherty,<sup>7</sup> which should be studied. Systematic studies have also been made in the author's own laboratories, continuously for the past ten years, dealing with the structure of the skins of different animals and the changes which they undergo during the conversion of the skin into leather. Most of the data presented in this chapter have either been obtained or confirmed by direct observation. The histological technic employed is described in detail in Volume II, to which reference should be made for further explanation of the information given under each photomicrograph.

### General Histology of Skin.

One of the most important functions of the skin is to keep the body temperature constant. It is supplied with a wonderfully delicate mechanism which controls the escape of heat from the body, using the evaporation of water to accelerate the heat escape, when necessary, and supplying an oil to the surface of the skin to retard the loss of heat when the external temperature falls. The skin is also an organ of sense, equipped with nerves sensitive to touch, pain, heat and cold. As an organ of secretion and excretion, it is supplied with glands, ducts, muscles, and blood vessels. It is a covering protecting the body against bacterial infection and acting as a buffer against shocks and blows. In strong sunlight, it is capable of developing color filters to protect the underlying tissues from the destructive action of the ultraviolet rays of the sun.

The many intricate functions of the skin are associated with a structure and chemical composition that are exceedingly complex and variable, depending upon the kind of animal, its age and habits, and even upon the location on the individual creature. Plates 1, 2, 3, and 4 all represent sections of human skin, but they are cut from the scalp, back, finger, and heel, respectively. A careful study of these plates will show how futile it would be to try to learn all about skin structure from the examination of a single specimen. In the section from the scalp, fat cells make up the greater portion of the whole, while in that from the back there are relatively very few fat cells, but a great abundance of fibers of connective tissue. In the section from the heel, fat cells and connective tissues are both very prominent, but no hairs are seen. In fact, there are many striking points of difference between all four types of skin.

The general histology of skin, covering structures common to a great many types of skin, will be discussed first and then the special structures of different types of skin will be described.

*Cells.* The tissues of which skin is composed either consist of cells or are the product of cells, which may be considered as the unit building stones of living matter. They are the factories in which the materials needed by the body are made and it is through their reproduction that the body lives, grows, and reproduces. Each cell consists of a nucleus suspended in protoplasm enclosed between very thin walls acting as a semi-permeable membrane. This general structure can be seen very clearly in the cells of the sebaceous glands pictured in Plate 16. In the upper left hand corner there are two groups of these glands.



The cell walls can be seen as a delicate network and the stained nuclei as dark dots.

Because cellular activity is the basis of life, it has been the subject of an enormous amount of study, but its molecular mechanism is so complex that it has baffled all attempts to explain it to the satisfaction of all investigators. Materials from the lymph and blood streams diffuse through the cell walls into the cell where they are converted into new materials more useful to the body or into materials to be thrown off or wasted.

The first operation in the reproduction of cells is the diffusion of nourishment into a cell through the membranous walls. This is followed by a period of growth. In the protoplasm, besides the nucleus, there is a small body known as a centrosome, which separates during the period of growth into two centrosomes which then repel each other. The nucleus contains a nucleo-protein body known as chromatin, which now separates into a number of unit segments called chromosomes. According to McLaughlin and O'Flaherty,<sup>7</sup> the number of chromosomes is characteristic of the species of animal from which the cell was obtained. The chromosomes finally split longitudinally and one half of each unit or chromosome then passes in the opposite direction toward a centrosome. In this manner each new daughter cell receives exactly the same number of structures as were found in the parent cell. These longitudinal halves then constitute the chromosomes of the daughter cells and gradually become mature structures, while the cell wall constricts forming two complete and separate cells.

*Tissues.* Seven general classes of tissues may be recognized in the skin: epithelial, connective, muscular, nervous, glandular, and fatty tissues and the tissues of the blood. These tissues either consist of aggregates of many individual cells or of material resulting from cellular activity. The epithelial tissues consist of layers of cells, which cover all the free surfaces of the animal body. The connective tissues are distinguished from the other fundamental tissues of the body by the fact that their cells lie imbedded in extracellular material which appears to be the result of their activity. The various types of connective tissues are distinguished from each other by the kind of extracellular tissue which they produce, such as bone, cartilage, etc. The muscular tissues have a well developed power of contracting, apparently without change of volume, the decrease in length being compensated by an increase in diameter. The cells of the striated or voluntary muscles are long in relation to their width and are marked with transverse bands, while those of the smooth or involuntary

muscles are spindle shaped, without transverse striations. The nerve tissues found in the skin are protoplasmic prolongations of cells lying in the central nervous system, or in the ganglia closely associated with that system. The glandular tissues are found in the sebaceous and sudoriferous glands and the fatty tissues either between the fibers of connective tissue of the true skin or in the adipose tissues forming the lower boundary of the skin.

The skin is divided sharply into two layers, distinct both in structure and origin: a relatively very thin outer layer of epithelial tissue, the epidermis, and a much thicker layer of connective and other tissues, the derma or corium. Raw skin, as an article of commerce, has also a third layer, the superficial fascia, known to the tanner simply as flesh and containing both adipose and areolar tissues. In keeping with the nomenclature of the leather trade, the word flesh will be used only in this connection, although in anatomy flesh really means muscle tissue. In life, the areolar connective tissues connect the skin proper very loosely to the underlying parts of the body. The derma lies between the superficial fascia and the epidermis.

In the preparation of skin for tanning, except in special cases, such as the tanning of fur skins, the flesh and the entire epidermal system must be removed intelligently and with extreme care, leaving the derma to be converted into leather. The epidermal system, flesh, and derma will be described in turn.

*Epidermal system.* The epidermis is made up of cellular strata originating from the ectoderm, the outer layer of the young embryo, and the derma is derived, independently, from the mesoderm, or middle layer. These two layers grow independently throughout life and differ materially in both chemical and physical properties. In Plate 2 the epidermis can be seen as a dark band forming the upper boundary of the skin and constituting only about 1 per cent of the total thickness. So far as its growth is concerned, the epidermis may be looked upon as a parasite, although it is a most important part of the body. It has no blood vessels of its own, but rests upon the upper surface of the derma and draws its nourishment from blood and lymph supplied by the blood vessels of the derma. It grows only through the reproduction of its own cells.

The portion of epidermis in contact with the derma is a layer of living epithelial cells, rather elongated in shape. In reproducing, each cell increases in height and then subdivides, forming two cells, one above the other. This process is going on continuously. As the older cells are pushed outward, food is no longer available for reproduction

and the cells gradually become flattened by dehydration and other changes, the protoplasm dries up, and the cells die. In the outermost layer, the cells are dry and scaly and are gradually worn away. This scaling is often very noticeable on the scalp in the form of dandruff.

Where the epidermis is very thick, as on the heel, the gradual transition which the cells undergo in their outward course gives the epidermis the appearance of having several distinct layers. The portion of the epidermis shown in the upper left-hand corner of Plate 4 is shown at a very much higher magnification in Plate 5. Now the several strata can be seen very plainly.

The layer marked E is the uppermost part of the derma and numerous protuberances of its surface, called papillæ can be seen extending upward into the epidermis, giving the boundary between epidermis and derma a serrated appearance. Because of the presence of papillæ this region of the derma has been called the *pars papillaris*, but it should be noted that these papillæ do not occur in all types of skin.

D is the Malpighian layer of the epidermis, or *stratum germinatum*, so called because its cells are at all times reproducing new cells. This layer is built up of several rows of living epithelial cells, whose nuclei appear in the picture as dark spots. Tiny fiber-like processes, often called prickles, pass from cell to cell, forming a sort of contact between them. Extending between these prickles, which look as if they were walls in section, are protoplasmic processes and it is supposed that food passes upward between the cells and waste from the upper layers downward. From this food the cells derive the nourishment necessary for reproduction. This layer contains no blood vessels, but very fine nerve fibers pass into it from the derma, forming a network between the cells and terminating in bulbous swellings or undergoing a gradual breaking up into nerve granules.

As the new cells are formed, the older ones are pushed outward where there is a diminishing supply of nourishment and the protoplasm of the cells gradually dries up. Upon staining, the cells then appear as though they contained coarse granules and form the layer shown at C, which, from its appearance, has been called the *stratum granulosum*. The cells also contain a pigment, which is at least partly responsible for the color of the skin. This pigment, known as melanin, is thought to be a derivative of hematin containing iron and sulfur. It is very concentrated in the skin of the negro and almost entirely absent from the skin of a blonde. Apparently the pigment is formed as a protection against strong sunlight, both for the skin and the underlying

tissues. The pigmented layer may thus be looked upon as a color filter. When the pigment-containing cells are collected in spots, they appear as freckles. The pigment in the negro skin is found in the deepest cells of the *stratum germinatum*, in the connective tissue cells of the upper part of the derma, and in the wandering cells of the lymph, found in the lymph spaces or between the cells of the epidermis or connective tissues. The pigment granules are found only in cells.

As the cells are pushed still further outward, the cell granules break down, yielding a material, called eleidin, which resists staining and gives the epidermis in this region a transparent appearance, from which it has derived the name *stratum lucidum*. This layer is shown at B.

The cells continue to undergo changes during their outward course, becoming drier and flatter, and finally form the very thick layer shown at A, the *stratum corneum*, in which the cells tend to break away from each other and to scale off. This layer is being worn away continually and is replaced by the newer cells from below. The corneous layer is a very poor conductor of heat and the waxy material usually present on its surface makes it water repellent. In the photomicrograph a duct can be seen taking a spiral course up through the corneous layer. This is the outlet of a sudoriferous or sweat gland seated in the derma. Its opening at the surface of the corneous layer is called a pore.

All of the strata noted above can be detected readily only where the epidermis is very thick. In sections of skin such as is used for making leather, there are relatively so few layers of cells in the epidermis that the apparent division is only into two layers, the *stratum germinatum* and the *stratum corneum*.

The independent growth of the epidermis and derma involves a number of important appendages of the skin. In the epidermal system, the reproduction of epithelial cells produces, not only the epidermis, but also the hair and the sebaceous and sudoriferous glands. These cellular structures are composed of proteins of the class known as keratins as distinct from the collagens and elastins of the derma. Where a portion of the epidermis is lost, through accident, it can be regenerated only by the surrounding epithelial cells spreading over the bare spot, by reproduction. The necessity for removing the epidermal system completely before tanning and without any injury to the derma makes the difference in chemical composition between the two systems a matter of great importance to the tanner.

In the class with hair belong also nails, claws, hoofs, scales, and feathers, which are all special growths of the epidermis. To the

naked eye, the hair appears to pierce the skin, but actually it does not do so. An examination of Plate 2 will show that the epidermis dips down into the body of the derma, forming a pocket, or follicle, in which the hair grows. The follicle is complex in structure because it is made up of the epidermal layers on the hair side and of the layers of the derma on the other. At its bottom, the follicle is penetrated by a projection coming from the derma and known as the hair papilla, which is supplied with both nerves and blood vessels.

A good example of a hair papilla is shown in Plate 6 in the hair bulb from the skin of a hog. The bottom end of the bulb appears like a pair of pincers with the jaws slightly open and facing downward. A similar structure may be seen in the hair bulbs of the scalp shown in Plate 1. Passing through the opening in the jaws into the large open space above and resembling a candle flame in shape is the papilla, which contains tiny nerves and blood vessels which supply nourishment. Lining the lymph space surrounding the papilla are numerous epithelial cells, which derive from the blood and lymph the nourishment necessary for reproduction. As new cells are formed, the older ones are pushed outward through the follicle, forming the hair. The rate of growth of the hair is determined by the rate at which the cells surrounding the papilla reproduce.

The newly formed cells of the hair, like those of the Malpighian layer of the epidermis, are very soft. As they are pushed upward, they become elongated in shape and harder. In forming the hair, they assume the shape of the follicle; if this happens to be curved, the hair will be curly. In the negro, the follicles often have a curvature of nearly 90 degrees, which accounts for the tightness of the curls.

In studying sections of skin, the author has noticed that either the sebaceous or sudoriferous glands, or both, are highly developed where the hair is curly. Apparently, the growing glands crowd and bend the hair follicle and any bending of the follicle will result in curly hair, since the hair is molded into the shape of the follicle. The development of these glands depends upon climatic conditions and undoubtedly is also hereditary.

The portion of the hair showing above the surface of the skin is called the shaft and the lower portion the root, which enlarges into a bulb at its lower extremity, where it is penetrated by the hair papilla. The shaft is made up of a central medulla, or pith, of rounded cells, containing eleidin granules, surrounded by a much thicker portion composed of long fibrillated cells, containing pigment, and enclosed by an outer layer of cells which become hardened in the form of over-

lapping scales. These scales, which give fur and wool their felting properties, open outward so as to resist the pulling out of the hair. Unless the lighting is properly adjusted and the magnification sufficiently great, the scales are not easily discernible. In Plate 7 may be seen the scales of a tiny piece of wool. The scales of one side and the shadows of those on the other both show because the wool was photographed with transmitted light. The same general structure can be seen on most hair, but it is not always so pronounced.

When a hair is shed, the epithelial cells left surrounding the hair papilla keep on multiplying and soon another hair is formed to replace the one shed. Baldness results from the failure of the blood vessels of the papilla to furnish the required nourishment or from the destruction of the epithelial cells in some other way. Any serious attempt to grow hair on a bald head must be accompanied by some means of introducing living epithelial cells into the hair follicles, of which there are something like a thousand to the square inch, and then of furnishing a continuous supply of nourishment.

In old age, pigment is no longer available for the hair cells and the new hairs, containing no pigment, appear gray in color. Hair containing pigment, however, may look white by reflected light, due to the presence of tiny air bubbles among the cells.

Each hair follicle is supplied with sebaceous glands with ducts emptying into the upper portion of the follicle. A group of these glands can be seen in Plate 2. They are lined with epithelial cells which secrete from the blood the materials required for the synthesis of the oils which they produce. When they become charged with oil, the protoplasm disappears and the cell breaks down, discharging the oil into the duct. New cells are continually being formed to replace the old ones. The oil is forced into the follicle, where it coats and lubricates the hair, and finally to the surface of the skin, which it softens and protects against the cold. In contact with air, this oil thickens to the consistency of car wax, to which it is related. When the ducts become clogged with dirt, the pressure behind them causes them to become distended, giving rise to blackheads. Sebaceous glands are sometimes found also in parts of the skin free from hair.

Attached to each hair follicle, just below the sebaceous glands, and extending obliquely upward through the derma, almost to the surface, is a bundle of nonstriated muscle tissue, known as the erector pili muscle. In Plate 2 one of these muscles forms a V with the hair follicle, and the sebaceous glands may be seen within the angle so formed. The nerves supplying these muscles are known as the pilo-

motor nerves. These muscles contract under the influence of emotions, such as fear, surprise, anger, or other disagreeable states, or in response to cold or grazing tactile stimuli. Among the commoner visible effects are the roughening of the skin called goose-flesh and the effect of the hair standing on end, very pronounced in a frightened cat.

The real purpose of the erector pili muscles is apparently to protect the body against sudden changes of temperature by their control over the operation of the glands; they seem to act as effectively as a thermocouple in a good thermostat. Their contraction puts a pressure on the glands which causes the cells to give up their oil to the hair follicle and, in the process, the cells are destroyed. The oil is then forced up through the follicle to the surface of the skin, where it tends to stop the action of the sudoriferous glands and the evaporation of water from the surface of the skin.

The sudoriferous or sweat glands are coiled sacs with spiral ducts leading to the surface of the skin. In Plate 4 several of these ducts can be seen winding up through the epidermis and terminating at the surface as pores. The sacs of the sweat glands are lined with epithelial cells, which are continuous with the cells of the Malpighian layer of the epidermis, and which secrete water, salts, urea, and other wastes from the blood and pass them out through the ducts. Where no sebaceous glands are present, the sudoriferous glands also provide an oily fluid to keep the surface of the skin soft. These glands serve the dual purpose of disposing of waste products and of permitting control of the body temperature through the regulation of the rate of evaporation of water.

This entire epidermal system, including the epidermis, hair, and sebaceous and sudoriferous glands, must be removed from the skin in such manner that the derma suffers no injury that can be detected in the finished leather.

*Flesh.* The skin is connected to the underlying parts of the body very loosely by means of areolar connective tissue, consisting of fibers of white connective tissue very loosely and irregularly arranged. Accompanying the areolar tissue in this region, there is usually a considerable amount of adipose tissue, so called because it is the seat of fat deposits, usually most numerous in the vicinity of the abdomen, which serve to protect the body against cold. Together these tissues constitute the bulk of what the tanner calls the flesh of the skin, the rest being made up of fibers of yellow connective tissue, blood vessels, and nerves.

The looseness of connection by the areolar tissues allows the skin

very free movement and, incidentally, makes flaying a much simpler operation than it would otherwise be. The flesh, while not a part of the skin proper, is of importance to the tanner because much of it remains adhering to the skins received at the tannery and must be removed prior to tanning. If left on the skins, it greatly impedes the progress of tanning.

Plate 8 shows a vertical section of the flesh adhering to the butt of a calf skin. The top quarter of the picture shows a portion of the derma bound on its under side by strands of elastin fibers, appearing as compact masses of black threads; actually they are of a pale yellow color. The fat cells of the adipose tissue are arranged in layers and are held together by fibers of white connective tissue. This region is sometimes supplied also with striated muscle tissue to permit the voluntary twitching of the skin; an example of this is to be seen on the under side of the kid skin shown in Plate 29.

The removal of flesh preparatory to tanning is an operation known in the tannery simply as *fleshing* and it is accomplished most efficiently when all of the tissues underlying the derma are cut away, leaving the derma itself entirely intact.

*Derma.* It is the derma, or true skin, that is actually used to make leather and the chief leather-forming constituent of the derma is collagen, the protein substance of the white fibers of connective tissue. Sound leather can be produced only from skins in which these fibers are well developed and abundant. The four contrasting structures shown in Plates 1, 2, 3, and 4 are typical of the extremes found in the skins of the lower animals. A skin composed chiefly of fat cells is of little value in making leather and one in which large groups of fat cells are interspersed between the collagen fibers will yield only a spongy leather because of the empty spaces left after the fat cells have been destroyed in the processes preparatory to tanning. The tendency toward one extreme or the other depends upon the location of the skin on the animal as well as on its species and its habits and feeding. In considering the general structure of skin, one should look upon the major portion of the derma as consisting of both fat cells and connective tissues, either of which may be very abundant or relatively scarce.

Unlike the epithelial tissues, the connective tissues are not made up chiefly of cells, but result from the activity of migratory cells very much smaller in size than the extracellular material which they produce. The relation of these cells to the collagen fibers of calf skin can be seen in Plate 9. The cells stain more deeply than the fibers



and appear in the picture as black specks having a diameter of about 1 millimeter, which means that the actual cells have a diameter of about  $\frac{1}{170}$ th of this. In the sections we have examined, the abundance of these cells diminished with increasing age of the animal.

By examining the cross sections of fibers running perpendicular to the plane of the page, the arrangement of the fibers, or fibrils, in bundles can be seen very plainly. Seymour-Jones<sup>9</sup> reasoned that the fibers were enclosed in very thin sheaths of what he termed "fiber sarcolemma." Later Turley<sup>10</sup> demonstrated the existence of such sheaths, using Bielschowsky's silver stain, and called them areolar tissue sheaths. The white fibers of the bundles were stained brown, while the sheaths were left unstained, appearing as refractile capsules surrounding the fiber bundles at some little distance away. The investigations of Wilson and Gallun<sup>11</sup> described in Chapter 9 seems to indicate that the surfaces of the collagen fibers are very much more resistant to tryptic digestion than the material just under the surface.

In contact with acid or alkaline solutions, the collagen fibers of the skin swell by absorbing some of the aqueous solution. When the fibers of fresh skin are thus swollen, they show constrictions at intervals, apparently due to relatively tiny encircling threads. Kaye and Lloyd<sup>4</sup> have recently given a description of this phenomenon, accompanied by very interesting photomicrographs of swollen fibers. These threads are thought to be made up of the protein reticulin, which will be mentioned again in Chapter 3.

The yellow fibers of connective tissue, composed of the protein elastin, are very much less abundant than the white fibers of collagen and also much smaller in diameter of cross section. There is usually a dense layer of elastin fibers at the lower surface of the derma, where it is attached to the areolar tissues, as shown in Plate 8, and another near the upper surface of the skin, in the region of the erector pili muscles. The great middle portion of the skin contains relatively few elastin fibers and these are generally to be found surrounding the blood vessels and nerves that traverse the derma.

The main trunk lines of blood vessels and nerves supplying the derma run parallel to the surface just above the lower elastin layer. From these trunk lines branches shoot upward and are distributed to all parts of the derma. A network of lymph ducts also is distributed throughout the skin.

Cross sections of the arteries and veins show three distinct layers: an outer layer of collagen and elastin fibers, a middle layer of non-striated muscle tissue and elastin fibers, and an inner membrane of

flattened cells. All three layers are pronounced in the arteries, but in the veins the inner layers are very much thinner than the outer layer. The walls of the veins are thinner than those of the arteries, which is responsible for the vein collapsing when emptied in contrast to the artery, which retains its shape. The veins are also equipped with semilunar valves, which prevent backflows of blood. A cross section of an artery can be seen at the top of Plate 8. It is the large circular body just to the left of the midline. To the right of the artery is a vein which has collapsed, because of its thin walls. The circular mass just under the artery is a cross section of a bundle of nerves. Three more sections of nerve bundles are prominent, elongated in shape, two just below the vein and one to the extreme left of the artery. A similarly located artery may be seen in the section of calf skin pictured in Plate 28.

In those parts of the body where the sense of touch is well developed, as in the fingers and bottoms of the feet, there are numerous protuberances of the surface of the derma into the epidermis, called papillæ. These may be seen clearly in the sections of finger and heel shown in Plates 3, 4, and 5. They are said to be of two kinds, one containing blood vessels furnishing nourishment to the active epithelial cells in their vicinity and the other containing, in addition to blood vessels, nerves sensitive to touch, pain, heat, and cold. The papillæ are usually arranged in definite patterns which do not change throughout life; the design of the thumb print is produced by papillæ. The epidermis above the papillæ is thinner than at other points, the papillæ serving the purpose of bringing the nerve ends nearer to those surfaces where they are most needed or where the epidermis is very thick. In some parts of the body the papillæ are either absent or so little developed as not to be easily recognizable.

The portion of the derma immediately in contact with the epidermis has been called the "grain membrane" by Seymour-Jones<sup>9</sup> because it forms the grain surface of the finished leather. Although its boundary on the side in contact with the epidermis is very sharp, on the other side it blends into the rest of the derma with no sharp change of properties. The fibers of connective tissue grow finer as they near the grain surface, in which the fibers are extremely fine and generally run parallel to the surface. They can be seen very plainly in the horizontal section of tanned calf skin shown in Plate 19. Whether or not the fibers of the grain surface are continuous with those of the connective tissues of the derma, they seem to possess somewhat different properties. When unhaired skin is kept in boiling water, the

fibers of the grain surface remain as a thin sheet, although somewhat changed, long after the larger collagen fibers below have passed into solution as gelatin. The outer surface is then very sharp, but the inner side, facing the remnants of the collagen fibers, appears jellylike and heterogeneous, indicating a gradual change in properties of the fibers as they pass from the derma into the grain surface.

It is of great importance that no damage be done to the grain surface in removing the epidermis, because it determines the appearance of the finished leather. It is therefore fortunate for the tanner that the fibers in this surface are more resistant to the action of alkalies than the epidermis above it and more resistant to the action of tryptic enzymes than the elastin fibers below it. The grain surface is readily attacked by proteolytic bacteria under certain conditions, however, resulting in what is known to the tanner as pitted grain. Damage of this sort is pictured in Plate 42 of Chapter 6.

Some evidence has been furnished to indicate that the grain membrane is separated from the *stratum germinatum* of the epidermis by an extremely thin film called the hyaline layer, although the existence of such a layer is denied by Küntzel.<sup>5</sup> Seymour-Jones<sup>9</sup> refers to such a layer in his writings. Upon staining some sections of steer hide, which had been unhaired after lining in simple lime for 8 days, with thionin or toluidine blue, Turley<sup>10</sup> noted a thin band, about 5 microns thick, of blue stained material on the outer skin surface. Later, while examining the details of collagen fibers stained with Bielschowsky's stain, he noted a colorless refractile membrane covering the fibers and also lining the hair follicles. The material seemed to be quite definite and not due to any optical effects. The staining results indicated that its composition is different from that of the fibers of the grain membrane. Moreover its composition seems to be different from that of the cells of the *stratum germinatum*, since it remains after these cells have been dissolved away by pure lime water, although Seymour-Jones<sup>9</sup> suggested that the effect giving the appearance of what is called the hyaline layer may actually be caused by the action of lime on the lower layers of the epidermis. Such a layer does not appear in stained sections of fresh skin, but might, of course, be masked by other constituents. Turley regards the evidence as pointing to the hyaline layer being identical with what histologists generally call the *basement membrane*.

The design of the grain surface, as seen on the skin after unhairing and tanning, is distinct for each species of animal, while the fineness of the pattern is an indication of the age of the animal. It is due to

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the arrangement of the hair follicles and pores, and of the papillæ where these are present. The grain surfaces of the tanned skins of a number of different animals are shown in Plates 10 and 11. They are all magnified to exactly the same extent and are directly comparable. It will be noted that the cow and calf have the same pattern, but that it is much coarser in the older animal. These designs can be used to identify different species of animal.

We shall now turn from considering the general histology of skin to the more detailed structures shown by definite types of skins used in making leather.

### **Cow Hide.**

In selecting skin for the production of heavy, sound and durable leather, the tanner usually chooses the hide of the steer or cow. In Plate 12 is shown a vertical section of cow hide taken from the thickest part of the butt. The specimen was fixed in Erlicki's fluid immediately after the death of the animal. This is the type of skin suitable for manufacture into sole leather or heavy belting or harness leather. Over 80 per cent of the total thickness of the hide is made up of heavy, interlacing bundles of collagen fibers, the chief leather-forming constituent of skin, and very few of the fat cells that tend to make the leather spongy are to be found among these fibers.

The epidermis appears as a thin, dark line forming the upper boundary of the section and occupying barely one-half of one per cent of the total thickness, the rest being the derma, the adipose tissue having been removed from this portion of the hide in flaying. The epidermis can be seen to dip down into the derma in many places, forming the follicles in which the hairs grow.

The presence of the muscles, glands and follicles in the top fifth of the derma give this region the appearance of a layer quite distinct from the lower part of the derma. Indeed, it is advantageous, in leather manufacture, to look upon the derma as divided into two distinct layers. The dividing line might conveniently be taken as that formed by the deepest points of the sudoriferous, or sweat, glands. The lower, fibrous region of the skin is often referred to as the reticular layer because of the network appearance of the collagen fibers. This name might well be accepted for most skins suitable for leather manufacture, although it might seem somewhat strained for skins in which the derma is made up largely of fat cells. The chief function of the upper layer seems to be that of a thermostat for the body and the

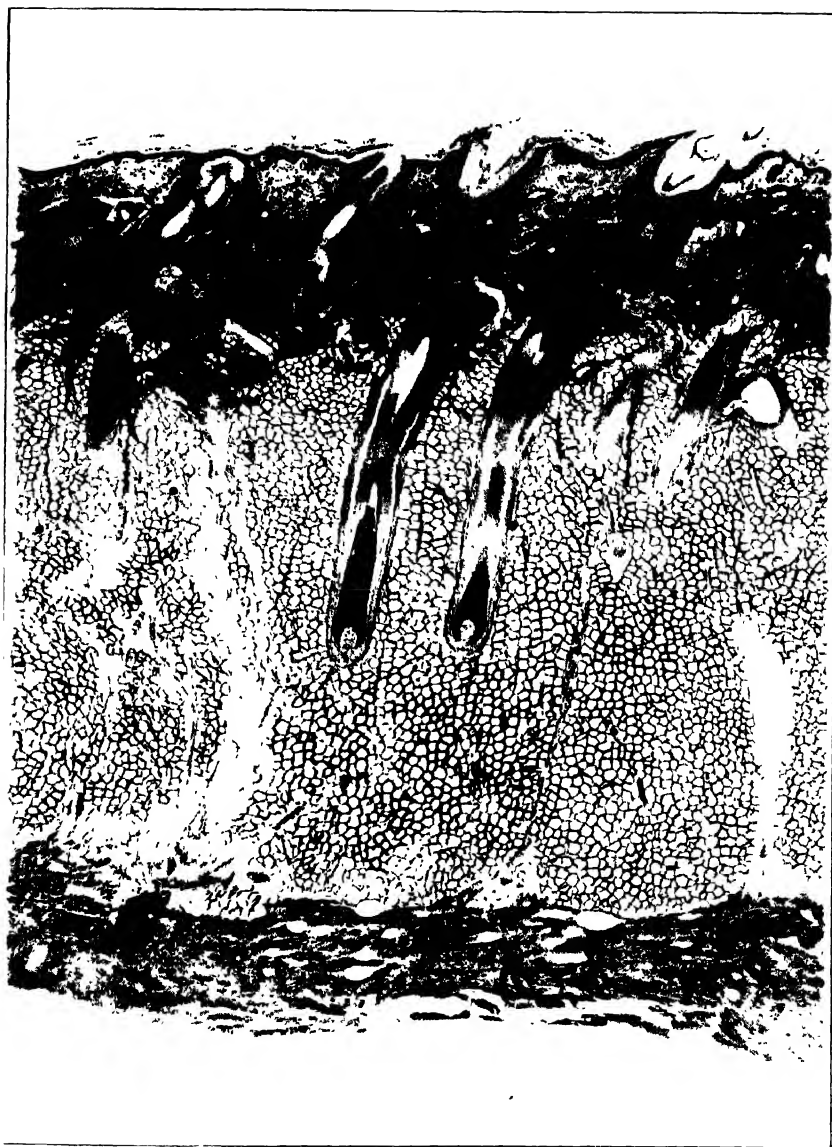


Plate 1.—Vertical Section of Human Skin.

Location: scalp.  
Thickness of section  $20\ \mu$   
Stains: Delafield's hematoxylin,  
Eosin

Eye-piece, none.  
Objective: 48-mm.  
Wratten filter: H-blue green.  
Magnification: 20 diameters.



**Plate 2.—Vertical Section of Human Skin.**

Location: lower part of back  
 Thickness of section: 20  $\mu$   
 Stains: Van Heurck's logwood,  
 Daub's bismarck brown.

Eye-piece: none  
 Objective: 32-mm  
 Wratten filter: H-blue green.  
 Magnification: 32 diameters.





Plate 3—Vertical Section of Human Skin.

Location finger  
 Thickness of section  $20\ \mu$   
 Stains Van Heurck's logwood  
 Daub's bismarck brown

Exepiece none  
 Objective 16-mm  
 Wratten filter H-blue green  
 Magnification 47 diameters



Plate 4.—Vertical Section of Human Skin.

Location: heel.

Thickness of section: 30  $\mu$

Stains: Delafield's hematoxylin,  
Eosin

Eye-piece: none.

Objective: 48 mm.

Wratten filter: H-blue green

Magnification: 20 diameters



Plate 5.—Vertical Section of Human Epidermis.

- A. Stratum corneum.
- B. Stratum lucidum.
- C. Stratum granulosum.
- D. Stratum germinatum.
- E. Pars papillaris.

Location: heel

Thickness of section: 30  $\mu$

Stains: Delafield's hematoxylin,  
Eos

Eye-piece: none

Objective: 8-mm

Wratten filter: A-red

Magnification: 100 diameters

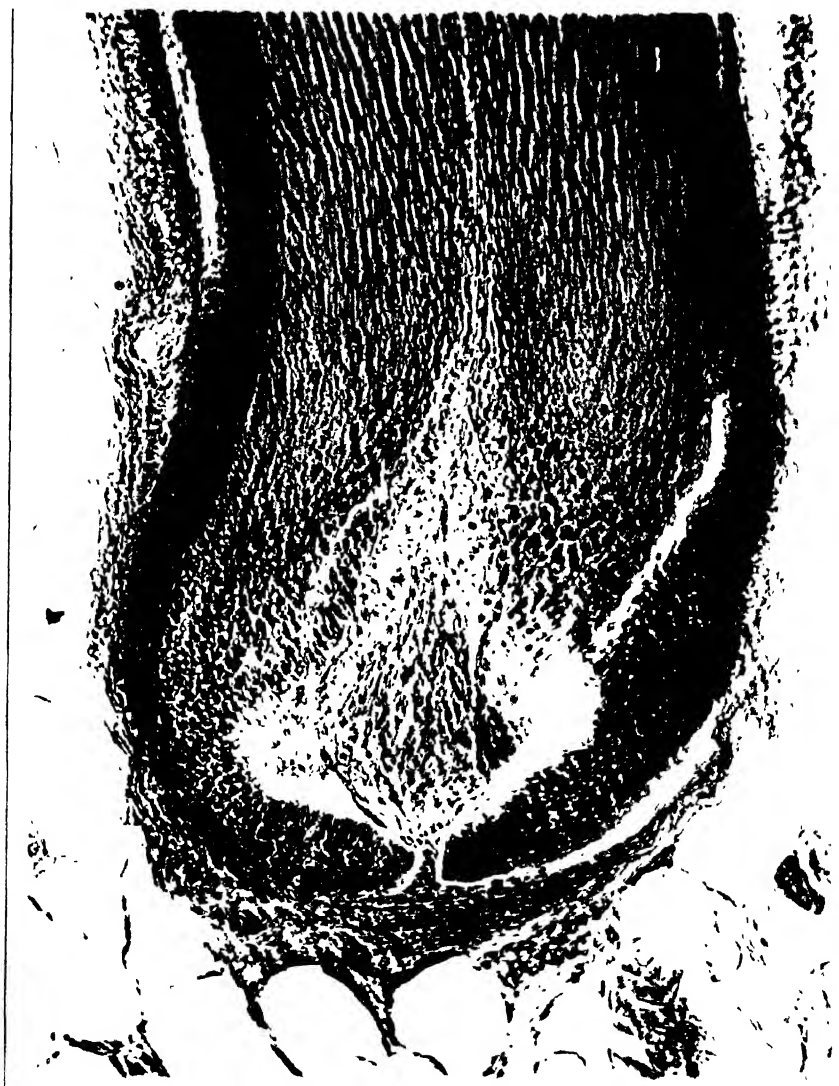


Plate 6.—Vertical Section of Hair Bulb from Hog, *in situ*.

Location: butt  
 Thickness of section, 20  $\mu$ .  
 Stains: Van Heurck's logwood  
 Picro-indigo-carmin

Eye-piece 5X  
 Objective 8-mm  
 Wratten filter F-red  
 Magnification 225 diameters



Plate 7.—Segment of Sheep Wool.

Stain none  
Eyepiece 7.5X.  
Objective 4-mm

Wratten filter: H-blue green  
Magnification, 1260 diameters

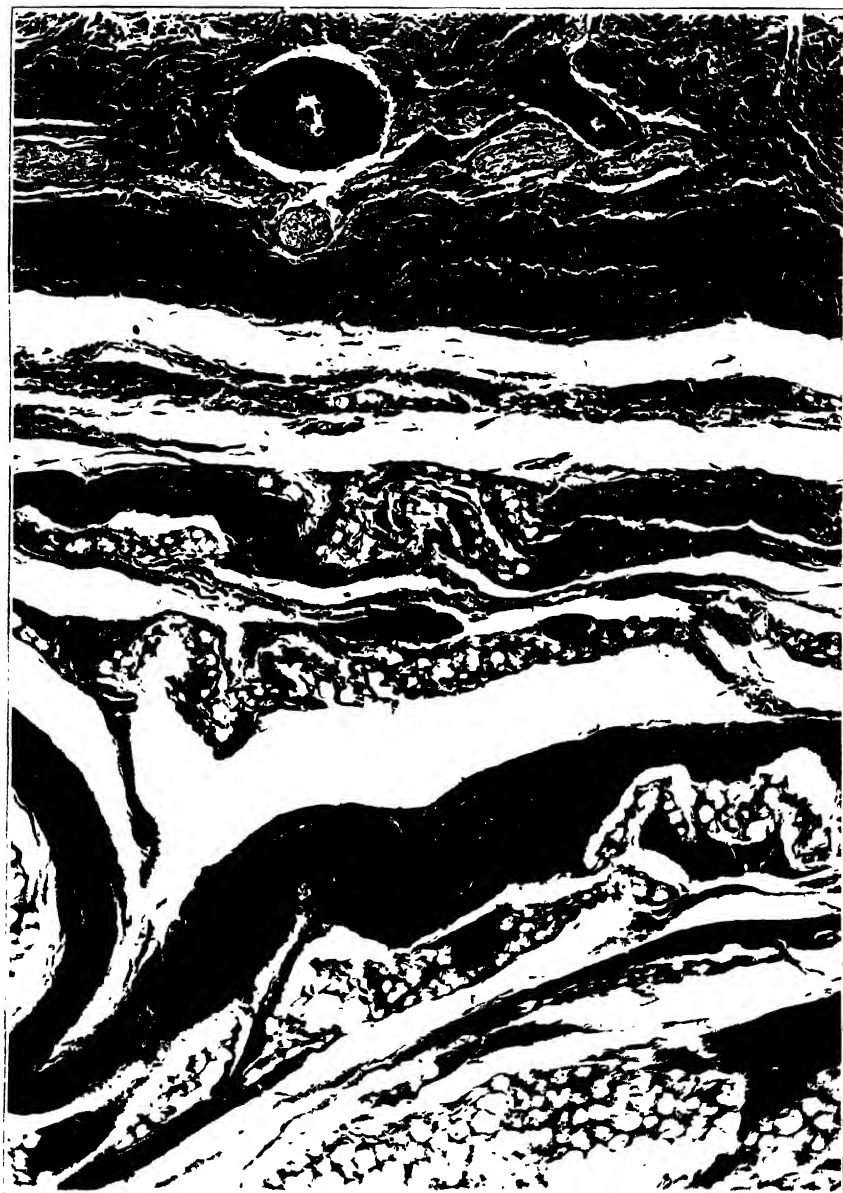


Plate 8.—Vertical Section of Calf Adipose Tissue.

Location: butt

Thickness of section  $20\ \mu$

Stains: Van Heurck's logwood,

Daub's bismarck brown

Excipiece: none

Objective: 16-mm

Wratten filter: H-blue green

Magnification: 70 diameters



**Plate 9.—Vertical Section of Reticular Layer of Calf Skin.**

Location, butt

Thickness of section  $20\ \mu$

Stains, Van Heurck's logwood,

Daub's bismark brown

Exepiece 5X

Objective 16-mm

Wratten filter B-green

Magnification 170 diameters.

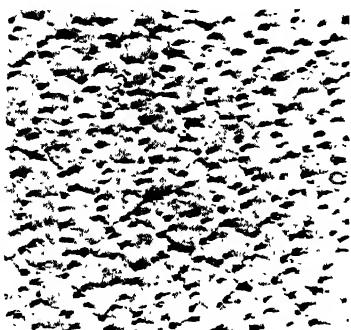


Fig. 1

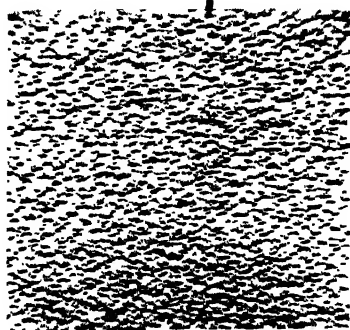


Fig. 2

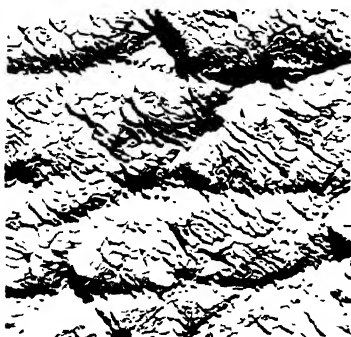
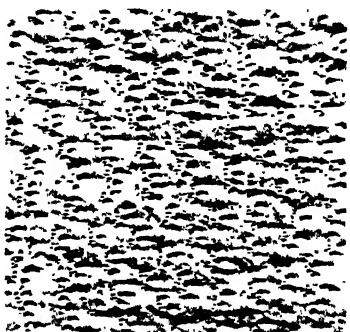
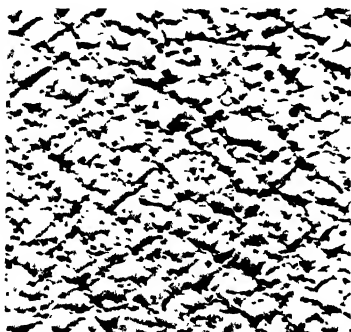


Fig. 3

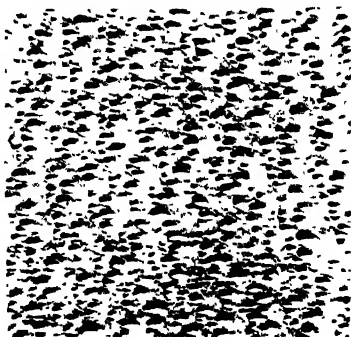


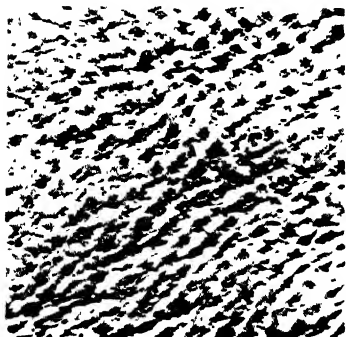
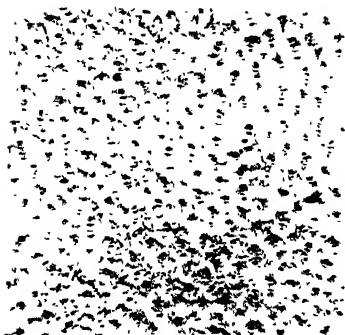
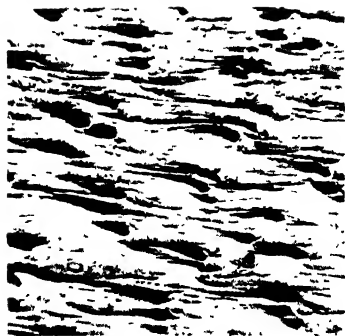
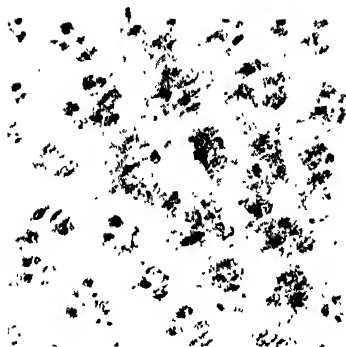
Fig. 4

Plate 10.—Grain Surfaces of Tanned Skins

Ex. piece none  
Objective 48-mm

Wlatten filter K2-yellow  
Magnification 7 diameters





Black cat

Black cat

Plate 11.—Grain Surfaces of Tanned Skins.

Eye-piece none  
Objective 48-mm

Wratten filter K2-yellow  
Magnification 7 diameters

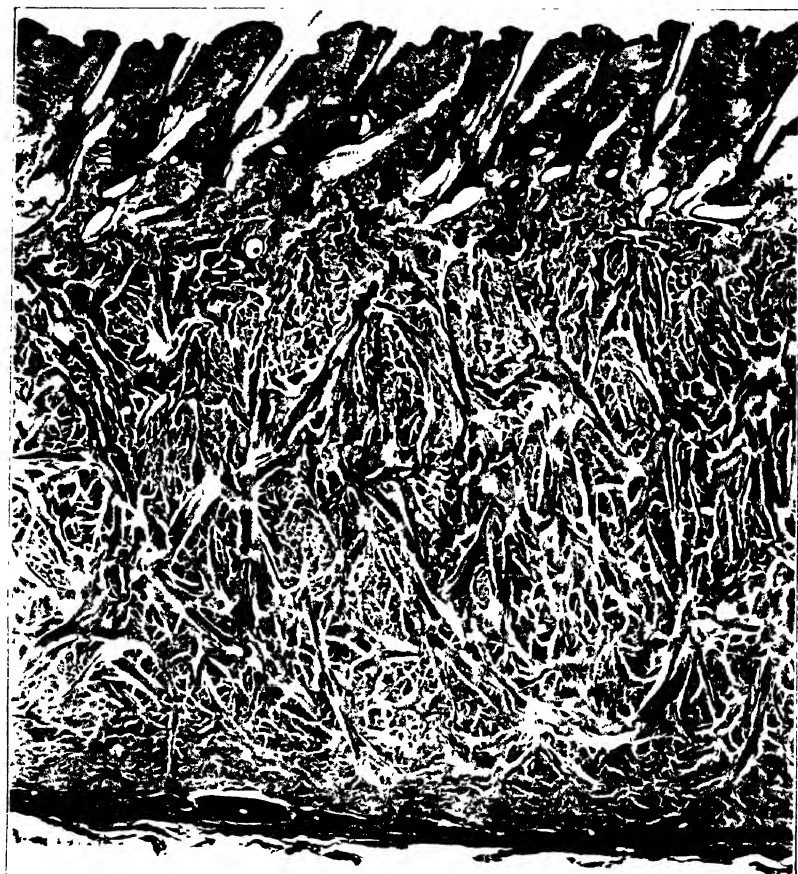


Plate 12.—Vertical Section of Cow Hide.

Location: butt  
 Thickness of section: 20  $\mu$ .  
 Stains: Van Heineck's logwood,  
 Daub's bismarck brown

Erypsice: none  
 Objective: 18-mm  
 Wratten filter: H-blue green  
 Magnification: 16 diameters.



Plate 13.—Vertical Section of Thermostat Layer of Cow Hide.

Location butt  
 Thickness of section  $20\ \mu$   
 Stains Van Heurck's logwood,  
 Daub's bismarck brown

Eye-piece 5X  
 Objective 16-mm  
 Wratten filter H-blue green  
 Magnification 75 diameters



Plate 14.—Horizontal Section of Cow Hide.  
(Through epidermis.)

Location: hind shank  
Thickness of section  $20\ \mu$   
Stains: Van Heurck's logwood,  
Daub's bismarck brown

Excisee: 5X  
Objective: 8-mm  
Wratten filter: H-blue green  
Magnification: 175 diameters



Plate 15.—Horizontal Section of Cow Hide.

(0.30 mm. below upper surface.)

Location hind shank

Thickness of section  $20\ \mu$

Stains Van Heurck's logwood,

Daub's bismarck brown.

Eye-piece 5X

Objective 8-mm

Wratten filter H-blue green

Magnification 175 diameters



**Plate 16.—Horizontal Section of Cow Hide.**

(0.51 mm. below upper surface.)

Location: hind shank

Thickness of section  $20\ \mu$

Stains: Van Heurck's logwood,

Daub's bismarck brown

Excisee: 5X

Objective: 8-mm.

Watten filter: H-blue green.

Magnification: 175 diameters



Plate 17.—Horizontal Section of Cow Hide.  
(0.81 mm. below upper surface.)

Location hind shank  
Thickness of section 20  $\mu$   
Stains Van Heurck's logwood  
Danb's bismarck brown

Exepiece 5X  
Objective 8-mm.  
Wratten filter H-blue green  
Magnification 175 diameters.

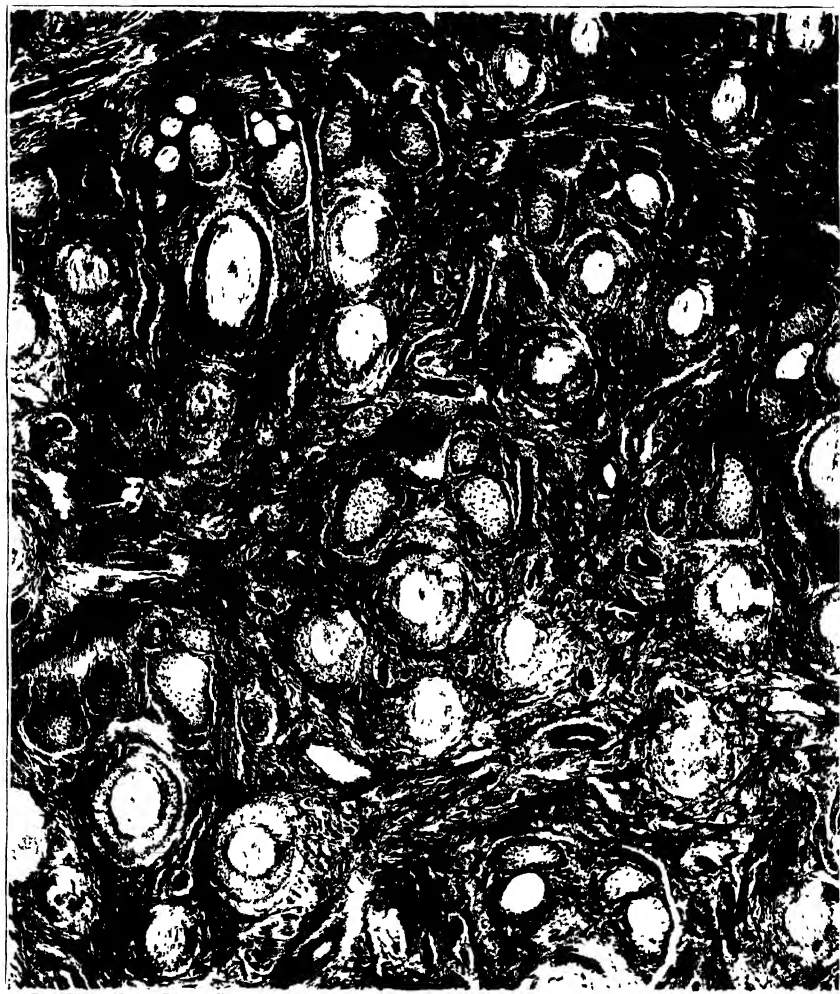


Plate 18.—Horizontal Section of Cow Hide.  
(0.54 mm. below upper surface.)

Location: hind shank

Thickness of section  $20\ \mu$

Stains: Van Heurck's logwood,  
Daub's bismarck brown.

Eye-piece none

Objective, 16-mm

Wratten filter H-blue green.

Magnification: 48 diameters.





Plate 19.—Horizontal Section of Calf Leather.  
(Through grain surface)

Location butt  
Thickness of section 15  $\mu$ .  
Stain indigo carmine  
Tannage vegetable

Eye-piece, 5X  
Objective, 8-mm  
Wratten filter F-red.  
Magnification 200 diameters

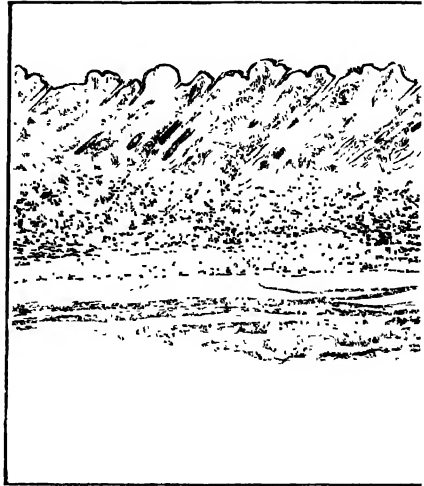


PLATE 20—Fore Shank.

PLATE 22—Neck

PLATE 21—Hind Shank

PLATE 23—Belly

### Vertical Sections of Calf Skin.

Locations, as noted

Thickness of sections:  $15\ \mu$

Stains: Van Heurck's logwood,  
Picro-indigo-carminic.

Embedder: none

Objective: 32-mm

Wratten filter: F-red

Magnification: 15 diameters

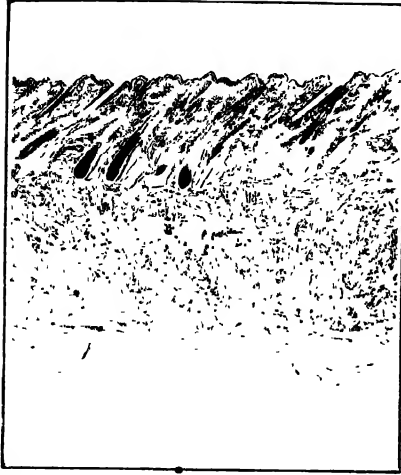


PLATE 24—Shoulder

PLATE 26—Butt

PLATE 25—Backbone.

PLATE 27—Tail.

#### Vertical Sections of Calf Skin.

Locations, as noted

Thickness of sections: 15  $\mu$

Stains Van Heurck's logwood,

Picro-indigo-carmine.

Eye-piece none.

Objective, 32-mm

Wratten filter: F-red

Magnification: 15 diameters.



Plate 28.—Vertical Section of Calf Skin.

Location butt  
 Thickness of section  $20\ \mu$   
 Stains Van Heurck's logwood  
 Daub's bismarck brown

Eye-piece 5X  
 Objective 32-mm  
 Wratten filter, H-blue green  
 Magnification 30 diameters

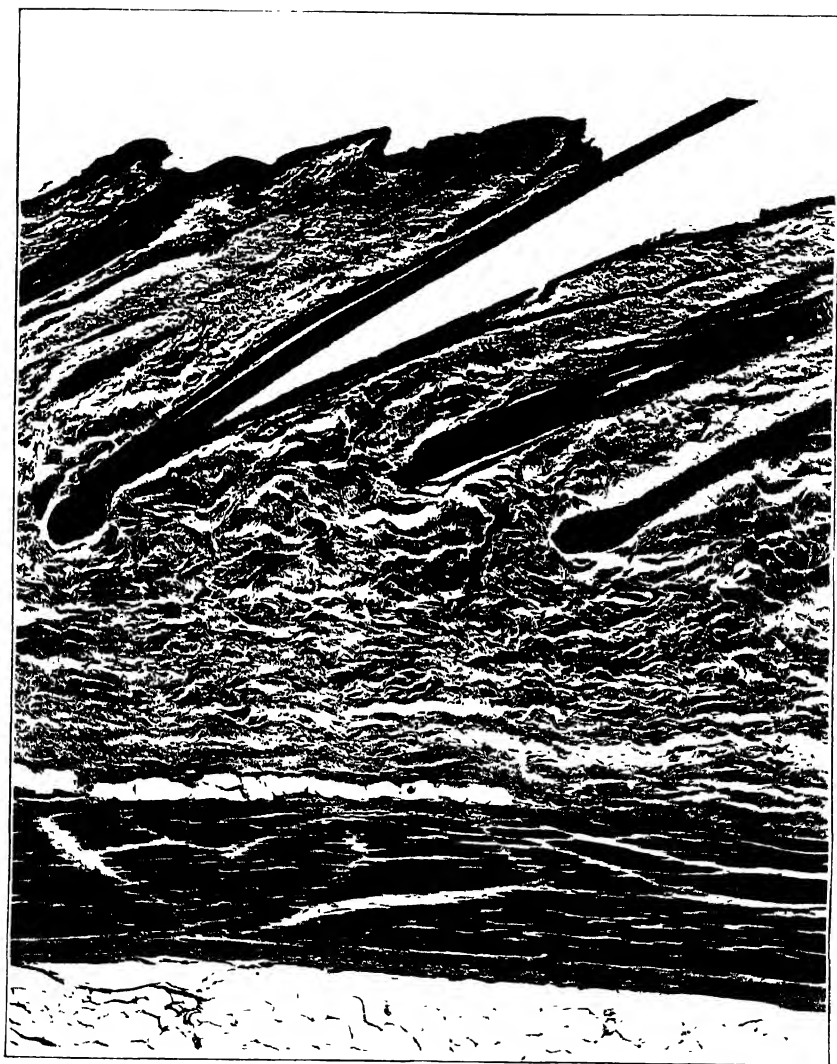


Plate 29.—Vertical Section of Kid Skin.

Location: butt

Thickness of section  $25\ \mu$

Stains: Van Heurck's logwood,  
Picro-indigo-carbime.

Eye-piece none

Objective 16-mm

Wratten filter H-blue green.

Magnification. 50 diameters.

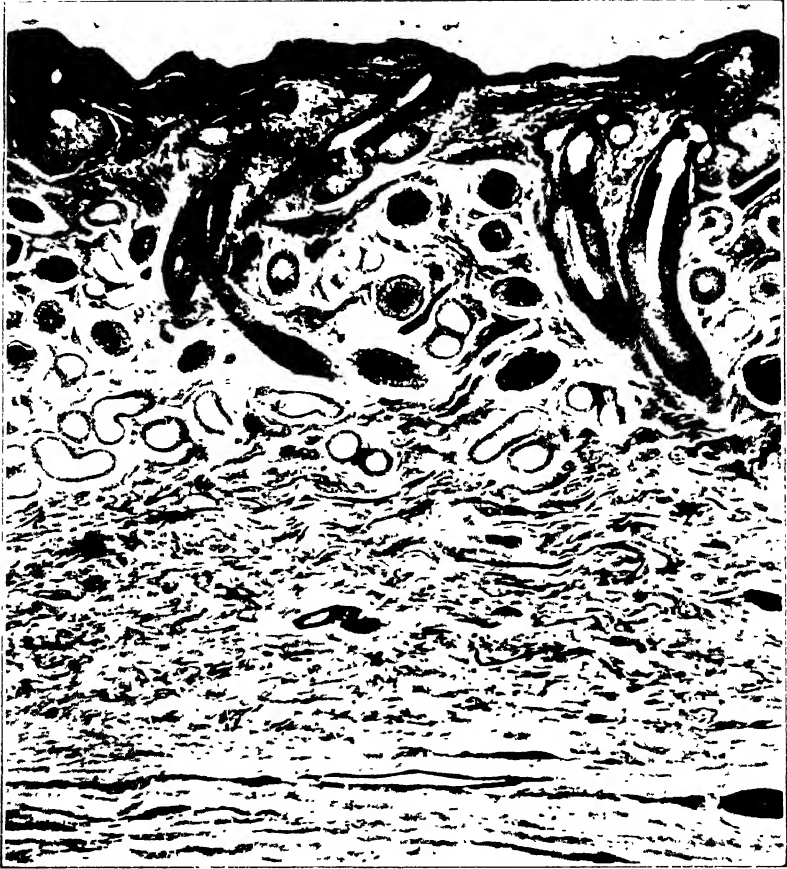


Plate 30.—Vertical Section of American Sheep Skin.

Location butt.

Thickness of section  $20\ \mu$

Stains: Van Heurck's logwood.

Daub's bismarck brown

Eye-piece none

Objective 16-mm

Wratten filter H-blue green.

Magnification 50 diameters



Plate 31.—Vertical Section of Chinese Sheep Skin.

Location butt  
 Thickness of section  $20\ \mu$   
 Stains Van Hemen's logwood,  
 Daub's bismarck brown

Lac piece none  
 Objective, 32-mm  
 Wratten filter H-blue green.  
 Magnification 25 diameters.

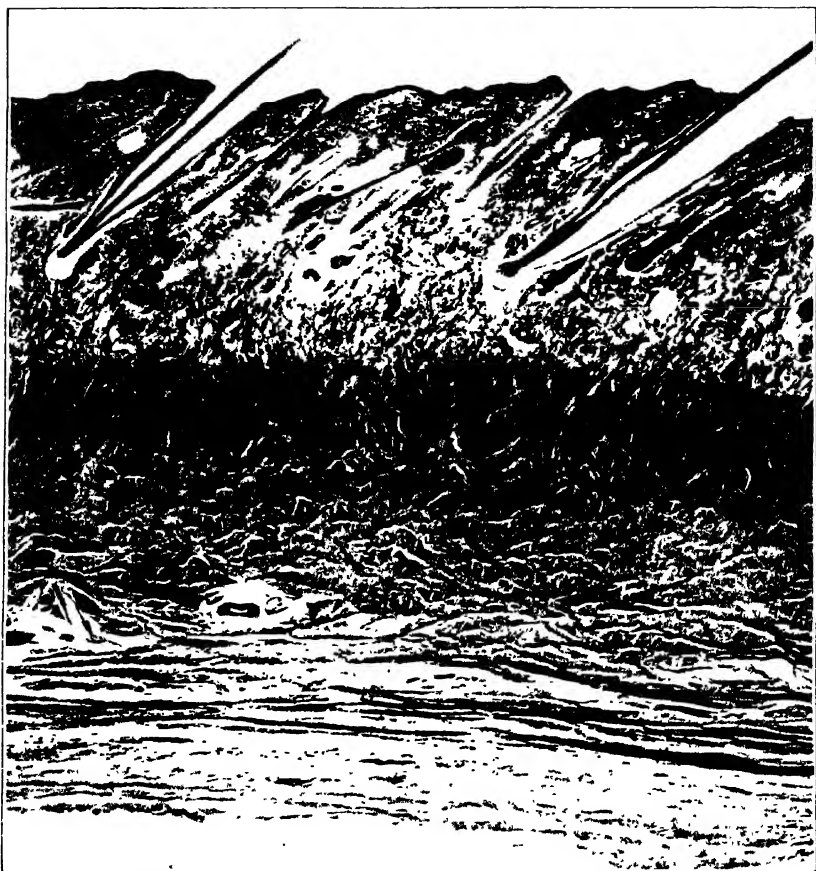


Plate 32.—Vertical Section of Horse Hide.

Location butt

Thickness of section  $20\ \mu$

Stains: Van Heurck's logwood,  
Daub's bismarck brown.

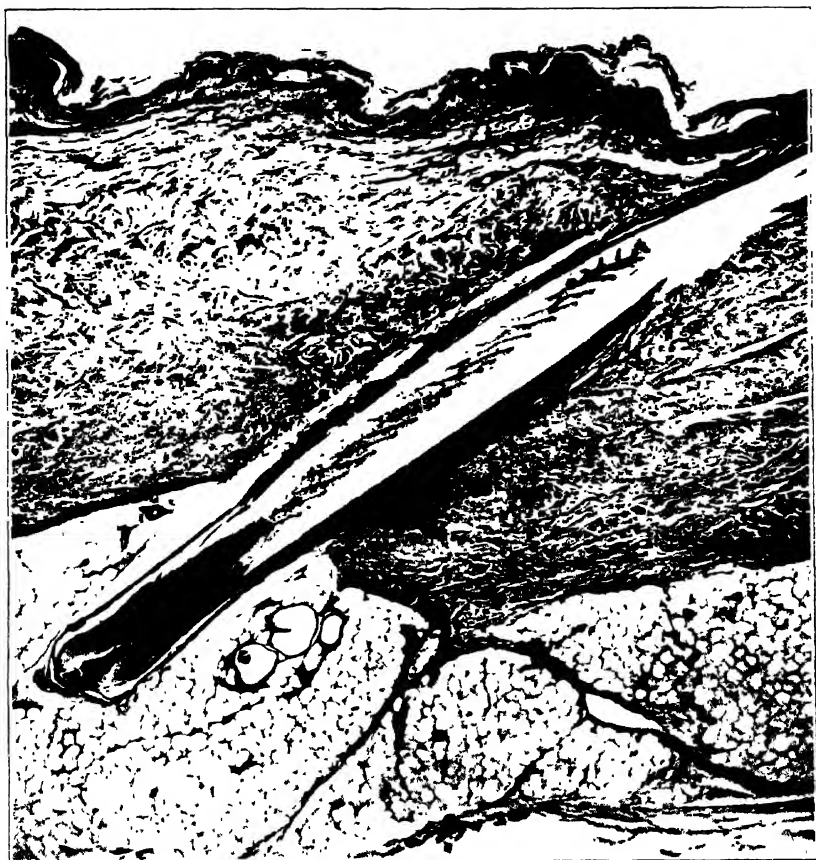
Eye-piece: none

Objective: 32-mm

Wratten filter: H-blue green

Magnification 20 diameters





**Plate 33.—Vertical Section of Hog Skin.**

Location butt  
 Thickness of section: 20  $\mu$   
 Stains Van Heurck's logwood,  
 Daub's bismarck brown.

Eye-piece: none  
 Objective 32-mm  
 Wratten filter: H-blue green  
 Magnification 20 diameters



Plate 34.—Vertical Section of Dog Skin.

Location: butt.

Thickness of section: 20  $\mu$

Stains: Van Heurck's logwood,  
Daub's bismarck brown.

Eye-piece: none

Objective: 32-mm

Wratten filter: H-blue green

Magnification: 25 diameters.



**Plate 35.—Vertical Section of Guinea Pig Skin.**

Location: butt

Thickness of section: 30  $\mu$

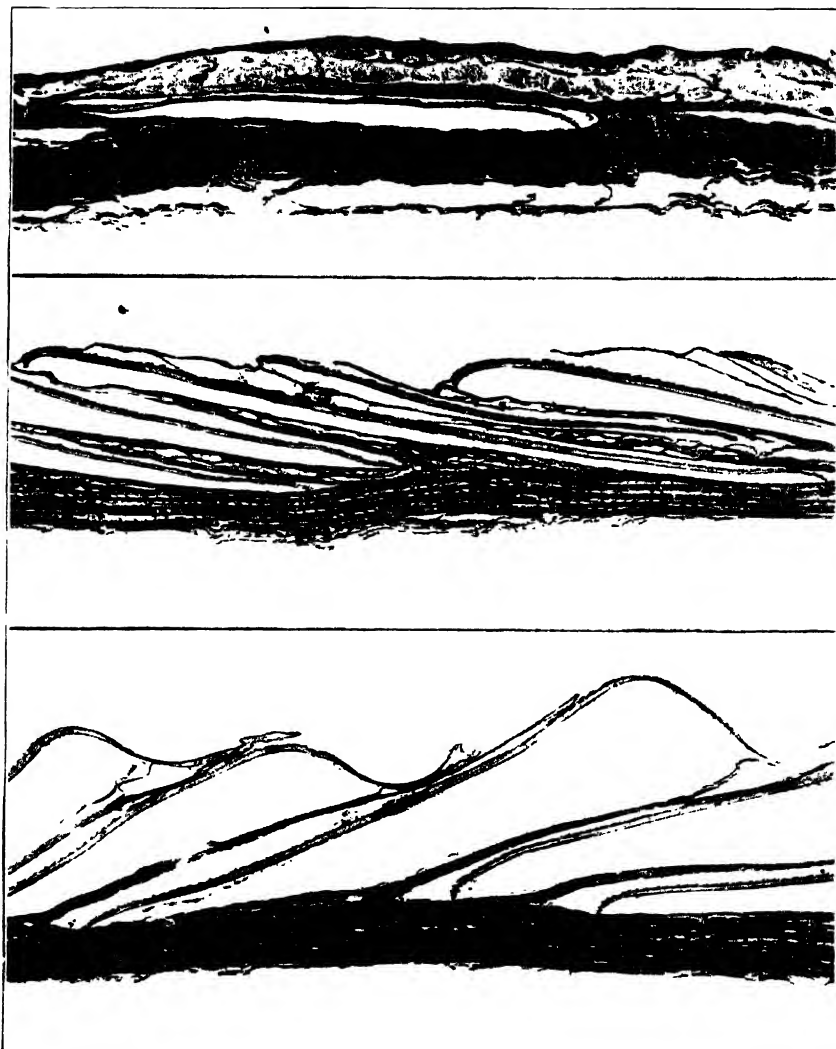
Stains. Van Heurck's logwood,  
Picro-indigo-carmin.

Eyepiece, none.

Objective: 16-mm

Wratten filter: F-red

Magnification. 70 diameters



**Plate 36.—Vertical Section of Halibut Skin.**

**Plate 37.—Vertical Section of Codfish Skin.**

**Plate 38.—Vertical Section of Salmon Skin.**

Location: side.  
 Thickness of sections: 20  $\mu$ .  
 Stains: Friedlander's logwood,  
 Picro-indigo-carmin.

Eye-piece: none.  
 Objective: 32-mm.  
 Wratten filter: H-blue green.  
 Magnification: 17 diameters.

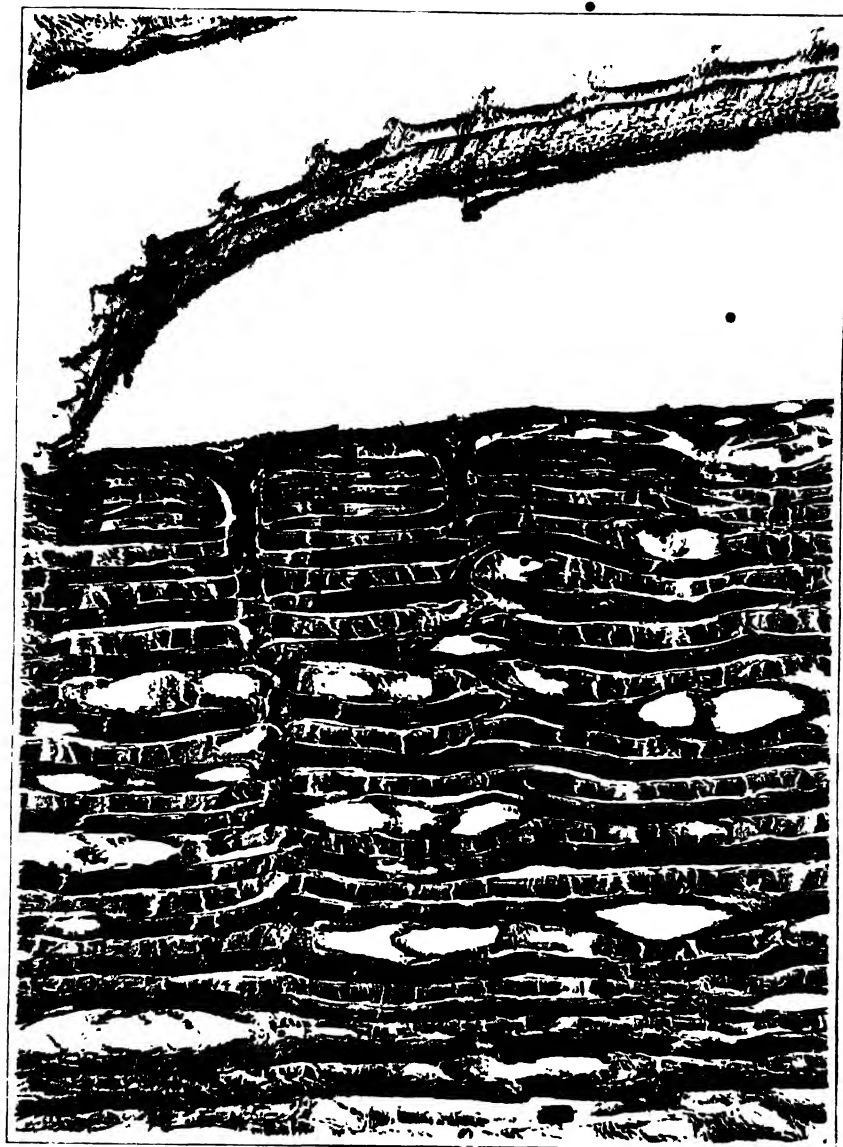


Plate 39.—Vertical Section of Salmon Skin.

Location: side.  
 Thickness of section: 20  $\mu$ .  
 Stains: Friedlander's logwood,  
 Picro-indigo-carbune.

Eye-piece: 7.5X.  
 Objective: 16-mm.  
 Wratten filter: H-blue green.  
 Magnification: 185 diameters.



Plate 40.—Cross Section of Angle Worm.

Location middle  
 Thickness of section 20  $\mu$   
 Stains: Van Heurck's logwood,  
 Daub's bismarck brown

Eye-piece none  
 Objective 48-mm  
 Wratten filter H-blue green.  
 Magnification 16 diameters.

writer, therefore, proposes the name thermostat layer as indicating its structure as well as its chief function.

In Plate 12 the thermostat layer occupies the top fifth and the reticular layer the remaining four-fifths of the section. The advantage of dealing with these layers separately is made clear by the fact that the structure of the reticular layer determines the physical properties of the leather such as tensile strength, solidity, resilience, etc., while the thermostat layer determines more particularly the appearance of the leather. In making the finer grades of leather, a great deal of attention must be paid to the thermostat layer. It is a matter of considerable importance that this layer is almost as thick in a small skin as in a large one; in the thinner skins and even in the thinner parts of the same skin, this layer occupies a greater proportion of the total thickness.

The section in Plate 12 is magnified only 16 diameters. In order to show the structure of the thermostat layer in greater detail, the third follicle from the left was magnified to 75 diameters. At this greater magnification, it is shown in Plate 13. The Malpighian and corneous layers of the epidermis can now be clearly differentiated, the latter becoming extremely thin where it lines the hair follicle. The *stratum granulosum* and *stratum lucidum* do not appear to be present in the epidermis. Attached to the base of the hair follicle and weaving its way upward to the right is the erector pili muscle. Just above this muscle and emptying into the hair follicle is a group of sebaceous glands. The empty space near the lower left-hand corner is that formerly occupied by a sweat gland whose duct has wandered out of the plane of the section, reappearing as a pore to the right of the hair just at the entrance to the hair follicle. The fine, black, threadlike lines running roughly parallel to the surface and to be found throughout the thermostat layer are the elastin fibers, or yellow fibers of connective tissue. In this layer, the collagen fibers are very much finer than in the reticular layer and appear to be broken up into individual fibrils. The grain surface appears only as portions of tiny fibrils with no sharp line of division from the rest of the derma. No papillæ are to be seen in this section; in fact, we found no papillæ in any part of the cow hide, except in the region of the legs.

In order to present a still clearer picture of the important thermostat layer, we prepared series of sections parallel to the surface of the hide. Strips of hide imbedded in paraffin were placed in the microtome and sections, each 20 microns thick, were cut in succession from the corneous layer to a point in the reticular layer, every section

being kept in order and mounted. The four horizontal sections shown in Plates 14 to 17 were prepared from a strip of hide taken from the hind shank so as to include the papillæ, which were not present in the other regions. Plate 14 shows a section cut through the epidermis. In the center is the opening of a hair follicle. The oval mass below the center is the cross section of a hair. The stringy lines forming an oval-shaped mass about the hair are the part of the corneous layer of the epidermis which dips down into the hair follicle. The heavy dots seen throughout the rest of the picture are the nuclei of the cells of the Malpighian layer of the epidermis. The irregularly shaped, light-colored patches are cross sections of the papillæ of the derma which protrude into the epidermis and are made up chiefly of nerves and blood vessels.

Plate 15 represents a section cut 0.30 millimeter below the upper surface of the corneous layer. It marks the plane of the derma where the ducts of the sebaceous glands empty into the hair follicles. In the center of the picture can be seen the cross section of the same hair as that shown in Plate 14 and of two ducts emptying into the follicle, just above the hair, to right and left. Both the ducts and the follicle are lined with epithelial cells which are continuous with the Malpighian layer of the epidermis and of which they are appendages. The dark, threadlike structures are elastin fibers. The larger collagen fibers of this region, being stained more lightly, are not prominent.

The section in Plate 16 forms the plane 0.24 millimeter below that of Plate 15. The hair whose cross section is shown in the lower part of the middle of Plate 16 is the same as that shown in Plates 14 and 15. The hair follicle at this point has a much thicker wall of epithelial tissues. Above the follicle, to the right and left, are the two groups of sebaceous glands whose ducts can be seen emptying into the follicle in Plate 15. These glands resemble bunches of grapes. Each dot is a cell nucleus and the fine lines are the thin walls bounding the cells. A portion of the erector pili muscle is visible at the midpoint of the top of the picture. It is passing obliquely upward through the plane of the section and away from the hair follicle. The contraction of this muscle exerts a pressure upon the cells and their oily contents are forced up through the ducts and into the hair follicles at the openings shown in Plate 15. Between the groups of glands and the hair follicles there is often found a mass of muscle tissue of the same kind as that constituting the erector pili muscle. Apparently the muscle extends also into this region and exerts its pressure upon the cells by a sort of pinching action.



Plate 18 is a photomicrograph of this same region taken at lower magnification so as to show the general arrangement of follicles and glands. The tendency for the hairs to group themselves in threes and fours is very noticeable. Some of the follicles are not so deeply seated as others and have their sebaceous glands in a plane higher up. This explains why no glands are to be seen in the vicinity of some of the follicles. The short, thick lines appearing here and there are arteries or veins wandering in and out of the plane of the section.

In Plate 17 is shown the section forming the plane 0.30 millimeter below that of Plate 16, or a total distance of 0.84 millimeter from the upper surface of the corneous layer. A cross section of the same hair as that shown in Plates 14, 15 and 16 appears in the center of the picture, but this time we have cut right through the hair bulb. To the right and left and above the hair bulb are the sweat glands. They appear as large, empty sacs, with portions of their linings of epithelial cells showing like leopard spots. In this plane the elastin fibers are much less numerous than in the regions higher up and the collagen fibers are now much larger and grouped in bundles. At a distance of 0.12 millimeter below this plane, we encounter the last of the epithelial cells of the sweat glands and therefore the lower boundary of the thermostat layer.

The reticular layer consisted almost entirely of collagen fibers, elastin fibers being present only in the lowest region and surrounding the blood vessels and nerves traversing other parts of the reticular layer.

### Calf Skin.

A calf skin, very naturally, appears much like a cow hide in miniature. In Plate 28 is shown a vertical section from the skin of a healthy young heifer calf, which had been fixed in Erlicki's fluid immediately following the slaughter and flaying of the animal. As a rule, the skin of a heifer calf has greater solidity and fineness of appearance than that of a steer calf and is, consequently, to be preferred for leather making. In comparing Plates 12 and 28, it should not be overlooked that the section of calf skin is magnified about twice as highly as that of the cow hide. In fact, in making comparisons of any photomicrographs in the book, erroneous conclusions may be drawn, if the magnifications are not taken into consideration.

The relatively greater thickness of the thermostat layer in the calf skin is noticeable. This fact is doubly interesting because the structure of this layer is of much greater importance for calf skin than for

cow hide; calf skins are generally used to make dressing and other leathers where fineness of appearance of the grain surface is highly valued, while cow hides more often are used for sole, belting, and harness leathers.

Another point to be noted in comparing Plates 12 and 28 is that the sections were cut from exactly corresponding parts of the skins of the two animals. The importance of this point will be made clear from a study of Plates 20 to 27. It is well known that a tanned skin is not uniform in structure throughout its entire area. The butt is

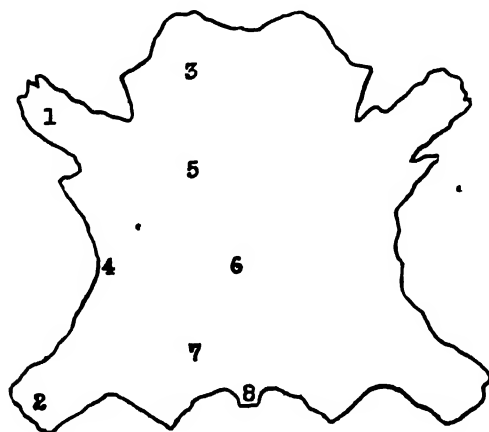


FIG. 1.—Diagram of Calf Skin showing locations of sections whose photomicrographs are shown in Plates 20 to 27.

- |                |                |
|----------------|----------------|
| 1: fore shank; | 2: hind shank; |
| 3: neck;       | 4: belly;      |
| 5: shoulder;   | 6: back bone;  |
| 7: butt;       | 8: tail.       |

usually much thicker and has greater solidity than any other part. The shanks are firm, but thin, while the flanks are thick, but spongy. In order to show how the structure of the skin varies in different regions, 8 strips were cut from the locations indicated in the diagram shown in Fig. 1. Vertical sections of these 8 strips are shown in Plates 20 to 27. In comparing the sections, it will be noted that the thickness of the thermostat layer is uniform throughout the skin, but that both the thickness and texture of the reticular layer vary widely.

The reticular layer is nearly 3 times as thick in the butt as in the hind shank. In the shoulder, the reticular layer is thinner than that of the butt and its fibers are somewhat finer. In the belly, the collagen fibers run nearly parallel to the grain surface and offer little resistance

to any tendency to pull them apart in a vertical direction, whereas many of the fibers in the butt run nearly vertically, with some running in almost any direction, making this region very resistant to distortion. The grain surface appears less serrated on the butt than elsewhere. In fact, most of the differences observable in the various parts of finished leather may be attributed to initial differences in structure of the living skin.

In studying Plate 28, use may be made of practically the entire description of cow hide given above. The bottom fifth of the picture shows the adipose tissue, consisting of rows of fat cells held together by strands of connective tissues. The thick band forming the lower boundary of the derma is closely interwoven with elastin fibers, but between this region and the thermostat layer, as in the cow hide, there are very few elastin fibers. Near the middle of the lower boundary of the derma is a cross section of an artery and further to the right a cross section of nervous tissue. The structure of the fibers of the reticular layer can be studied better in Plate 9, where they are more highly magnified.

### Sheep Skin.

Plates 30 and 31 show vertical sections of the skins of healthy sheep native of America and China, respectively. A comparison of Plates 30 and 28 indicates very plainly why sheep skin cannot be substituted for calf skin, where firmness and substance are desired. The collagen, or leather-forming, fibers of the sheep are extremely thin and not closely interwoven and tend to run parallel to the skin surface, which in itself makes for looseness of texture. Moreover, in the thermostat layer there are numerous sweat glands and fat cells, which leave empty spaces in the finished leather and make it very spongy.

It will be noted that the hair follicles are curved; this is responsible for the curliness of the wool. The hairs are virtually molded in the follicles whose shapes they assume. The hair of the calf is straight because the follicles are straight. In China, it is common practice to use male goats to guard the sheep and there is much interbreeding, but the progeny have straight hair. On a trip to China, Mr. Charles B. Simmons, Jr., secured for the writer a specimen of one of these straight-haired sheep, quite typical of the great Chinese herds. A section of this skin is shown in Plate 31; as expected, the follicles proved to be relatively straight. In the American sheep, we were unable to find one follicle lying wholly in a single plane. This made the study of the thermostat mechanism of the sheep more difficult

than with the calf, but they were found to be similar, that of the sheep merely being complicated by the twisting of the follicles. In comparing Plates 30 and 31, it should be noted that the magnifications are not the same.

The proportion of fat cells to collagen fibers in sheep skins varies considerably according to the feeding of the animal, and there is often to be found an almost continuous layer of fat cells separating the two main layers of the skin. In such cases, it is desirable to separate the skin into its two layers before tanning and to tan each separately rather than to try to keep them together. Usually the skins are split into two parts after the liming process and the thermostat layers, called grains, are tanned with sumac or other tanning extract to make leather suitable for bookbinding, hat bands, etc., while the reticular layers are converted into chamois leather, for which they are particularly suitable, by means of a tannage with cod oil.

Sections from the American sheep skin shown in Plate 30 were also taken at different stages of the tanning process and are shown in Volume II. They should be examined in connection with the study of the raw skin.

### Goat Skin.

In many respects the skin of the goat may be regarded as having a structure intermediate between that of the calf and the sheep. The fibers are fuller and firmer than those of the sheep, but are hardly equal to those of the calf. The glands and fat cells, which are responsible for the sponginess of sheep leather, are very much less abundant in goat skin, although it must be admitted that this is largely dependent upon the animal's feeding. Both the goat and the sheep skins of the general market vary widely in quality and substance, a fact which warrants a considerable extension of the study of their structures. Calf skins, on the other hand, do not vary in quality nearly so widely.

Like the calf, this goat has straight follicles, and, consequently, straight hair. The surface of goat skin is very much coarser than that of calf skin. A glance at Plate 10 will show that the pattern of the calf grain is considerably finer, even than that of the kid. Roughness of grain, however, is sometimes desirable and the grain surface of goat skins is often made still coarser by mechanical means.

A vertical section of kid skin is shown in Plate 29. This was just an average domestic skin in the condition in which fresh skins are usually received at the tannery. The epidermis is the very thin dark

line forming the upper boundary of the skin. It dips down into the derma, forming a nearly straight follicle, in which the hair grows. The erector pili muscle is the thin line running upward to the right from the base of the follicle. The opening of the sebaceous glands into the follicle can be seen just above the erector pili muscle. The fact that the collagen fibers run nearly parallel to the surface gives this skin, in its most solid part, a softness and looseness found only in the flanks of the calf skin.

Bounding the lower surface of the derma is a layer of striated muscle tissue, which permits the animal to twitch its skin. Muscles of this kind are often found on most of the various kinds of skins used for making leather.

A typical section of glazed kid leather used for shoe uppers is shown in Volume II. It is interesting to compare its general structure with those of the calf and sheep.

### Horse Hide.

The outstanding peculiarity of horse hide lies in the reticular layer. In the region of the butt there is a dense mass of collagen fibers in the reticular layer so compact as to render leather made from the butt naturally waterproof and nearly air tight. A section of horse hide taken from the butt is shown in Plate 32. The dense mass of fibers, often called the glassy layer, can be seen running horizontally across the middle of the picture and appearing much darker than the remaining fibers. The portion of the hide containing the glassy layer is known as the shell and is used to make the leather sold under the name of cordovan. The rest of the hide not only does not have this glassy layer, but the fibers of the reticular layer are very loosely interwoven, giving the leather made from it a spongy substance that limits its use.

The thermostat layer of horse hide resembles that of cow hide. The general arrangement of the hair follicles, the erector pili muscles, and the sebaceous glands can be seen in Plate 32, but the full detail shown in the sections of cow hide is lacking because the specimens of horse hide were not fixed immediately after the death of the animal, as in the case of the cow hide. The section, however, represents a hide in probably the usual condition in which horse hides are received at the tannery.

Plates in Volume II show a comparison of leather made from the shell and that made from the portion of hide immediately adjoining

the shell. In splitting the leathers to a nearly uniform thickness, the knife of the splitting machine cuts through the lower part of the glassy layer. The greatest contrast between the two specimens is thus shown in the lower portions.

### Hog Skin.

The comparatively low value of hog skin for leather manufacture can be appreciated by studying the section shown in Plate 33. The reticular layer is composed chiefly of fat cells, which have practically no value in making leather. We have here a case where the general use of the term reticular is apt to be misleading. The fat cells extend even up into the thermostat layer. The close relation of this structure to that of the human scalp, shown in Plate 1, should be noted.

The epidermis, as well as the upper surface of the derma, is very rough and irregular in appearance. As in other skins, the epidermis dips down into the derma, forming the follicles in which the hairs, or rather bristles, grow. The hair bulbs are imbedded in the mass of fat cells which make up the reticular layer. These fat cells extend higher up into the thermostat layer in the region of each hair follicle, about which the fat cells form cone-shaped masses. The structure of a hair bulb from the hog is shown in Plate 6.

The erector pili muscle belonging to the follicle shown in Plate 33 did not lie in the plane of the section. A portion of one of these muscles can be seen in Plate 94 of Chapter 10, which, because of its very much higher magnification, also shows the arrangement of the elastin fibers of the thermostat layer. The hog has relatively much fewer elastin fibers than the cow, calf, or sheep.

The roughness of the surface of the derma is further accentuated by the presence of papillæ, which seem to be rare in the skins of most of the lower animals studied. In the cow hide, papillæ were found only in the region of the legs, while in the calf, sheep, and goat skins, no papillæ were found at all. It would be interesting to determine whether the abundance of papillæ makes the hog more sensitive to touch and pain than the other lower animals. The extreme roughness of the grain surface of tanned hog skin is very noticeable in Plate 10.

After the skin has been unhaired and prepared for tanning, only a portion of the thermostat layer remains. The follicles then are simply pockets lined with the grain membrane, the lower portions protruding out from the under side of the skin. When the tanned skin is shaved down on the under side to make it smooth, the bottoms of these pockets are cut away, leaving holes wherever there were bristles in

the original skin. This serves further to lower the value of leather made from hog skin. A section of tanned hog skin is shown in Volume II.

### Dog Skin.

Plate 34 shows a section from the butt of a dog skin. This particular dog was a mongrel, and its skin probably represents only one of many types of dog skins. It is interesting because of its resemblance to the hog skin. The reticular layer is made up almost entirely of fat cells which also line some of the hair follicles nearly to the surface of the skin. Of interest also is the irregularity of the surface of the skin. Such a skin has a very low value for leather making.

It is not yet clear just what determines the selection of either fat cells or leather-forming fibers in the building of skin, although it is probably a matter of diet. Some skins have reticular layers composed of both collagen fibers and fat cells in almost equal amounts. Leather made from such skins is very spongy.

### Guinea Pig Skin.

A section of guinea pig skin is shown in Plate 35 as an example of very small skins. Such skins can be made into fairly good leather, but their diminutive size limits the demand for them and it is questionable whether such leather could be sold at a profit. A point worthy of note is that the thermostat layer of the guinea pig skin is of practically the same thickness as that of a calf skin, which is very much larger. As shown in the description of the different parts of the calf skin, when nature provides a thinner skin, she does so almost entirely at the expense of the reticular layer, and not of the thermostat layer. It is possible that a minimum thickness for any size of animal is required for the proper operation of this important layer.

The corneous layer of the epidermis appears like a few strands of delicate threads just above the Malpighian layer, the dark line bounding the upper side of the derma. The collagen fibers of the reticular layer are so fine that they appear only as thin threads even at a magnification of 70 diameters. The dark band crossing the bottom of the picture is a mass of striated muscle tissue.

### Ordinary Fish Skins.

The detailed structure of fish skins is very different from those of mammals. Nevertheless fish skins yield a leather comparing favorably

with some of the more common types of commercial leathers. Fish leather is very tough, as a rule, and is suitable for many purposes where great strength is required. Sturgeon leather used for lacing heavy belts together has been known to outwear the belts. It is said that the people of New England, in the old days, made shoes and gloves from the skin of the cod fish. Other fish skins are sometimes used for making fancy leathers.

In Plates 36, 37, and 38 are photomicrographs of sections of the skins of the halibut, cod, and salmon. These skins are covered by a thin epidermis which dips into the derma here and there forming follicles in which the scales grow. The scales of the fish correspond to the hairs of the warm-blooded animals. The scales may be recognized by their saw-tooth edges.

A portion of the right hand side of Plate 38 is shown in Plate 39 at a very much higher magnification so as to show the detailed structure of the derma. The upper portion of the picture is occupied by the lower end of a scale. We have not yet identified in fish skin the machinery of a thermostat layer like that common to the skins of mammals and, being cold-blooded, they probably have none. Instead of interlacing bundles of collagen fibers, ribbons of collagen running parallel to the surface make up the major portion of the skin. These ribbons do not interlace, but here and there we note bands of collagen running vertically through the skin. This adds greatly to the strength of the skin and prevents the distortion made possible in a vertical direction where all the fibers or ribbons run horizontally.

A section of tanned salmon skin, with the epidermal system completely removed, is shown in Volume II. This leather is purposely shown in the unfinished state because the structure is thus shown more clearly. In finishing such leather, either the loose, upper portion is rolled out smoothly and coated with a finishing material or it is shaved off and the under portion is treated with a suitable finish and embossed or plated.

### Other Skins.

Plate 40, showing a cross section of an angle worm, with its extremely tiny skin, is added merely as a curiosity. The epidermis is the outermost band, appearing black in the picture. At higher magnification, it appears as a honeycomb work of elongated cells next to the derma and as dried scales near the outer surface. The derma is made up of thin fibers generally running parallel to the outer sur-



face. A single hair appears in the section; it is one of a series used in locomotion.

Other interesting structures are to be found in the tanned skins of the shark, alligator, horned-toad, sea lizard, spotted ray, walrus, hippopotamus, camel, Persian lamb, kangaroo, slink calf, and shark, shown in Volume II.

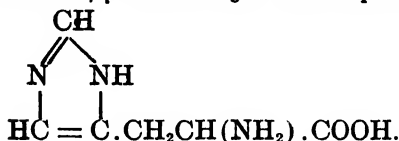
## Chapter 3.

### Chemical Constituents of Skin.

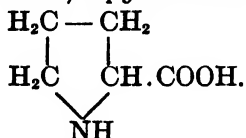
By far the greater portion of the solid matter of the skin consists of protein matter. The proteins form one of the most important and complex groups of organic compounds and are remarkable for the number of general physical and chemical properties which they possess in common and the extreme difficulty of making quantitative separations of the several members of any one group. They all contain carbon, hydrogen, nitrogen, and oxygen, and many of them also contain sulfur and phosphorus. They are all amphoteric, combining with both acids and bases, and those that do not dissolve in water swell by absorbing water. They are more or less readily hydrolyzed by boiling acid or alkaline solutions or by appropriate solutions of enzymes. Hydrolysis proceeds in steps yielding in turn bodies of decreasing complexity, the proteoses, peptones, polypeptides, and finally simple amino acids. Amines and ammonia are often found among the various hydrolytic products. The following amino acids have been isolated and identified from the hydrolytic products of different proteins:

1. Glycine, *aminoacetic acid*,  $\text{NH}_2\cdot\text{CH}_2\cdot\text{COOH}$ .
2. Alanine,  *$\alpha$ -aminopropionic acid*,  $\text{CH}_3\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
3. Valine,  *$\alpha$ -aminoisovaleric acid*,  $(\text{CH}_3)_2\cdot\text{CH}\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
4. Leucine,  *$\alpha$ -aminoisocaproic acid*  $(\text{CH}_3)_2\cdot\text{CH}\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
5. Isoleucine,  *$\alpha$ -amino- $\beta$ -methyl- $\beta$ -ethylpropionic acid*,  $(\text{CH}_3\cdot\text{CH}\cdot\text{C}_2\text{H}_5)\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
6. Phenylalanine,  *$\beta$ -phenyl- $\alpha$ -aminopropionic acid*,  $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
7. Tyrosine,  *$\beta$ -parahydroxyphenyl- $\alpha$ -aminopropionic acid*,  $\text{HO}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
8. Serine,  *$\beta$ -hydroxy- $\alpha$ -aminopropionic acid*,  $\text{CH}_2(\text{OH})\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .
9. Cystine, *di-( $\beta$ -thio- $\alpha$ -aminopropionic acid)*,  $\text{HOOC}\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$ .

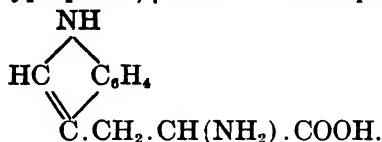
10. Aspartic acid, *aminosuccinic acid*,  $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ .
11. Glutamic acid,  $\alpha$ -aminoglutaric acid,  $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ .
12. Arginine,  $\alpha$ -amino- $\delta$ -guanidinevalerianic acid,  $\text{HN} : (\text{C} \cdot \text{NH}_2) \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ .
13. Lysine,  $\alpha$ - $\epsilon$ -diaminocaproic acid,  $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ .
14. Hydroxylysine,  $\alpha$ - $\epsilon$ -amino- $\beta$ -hydroxycaproic acid,  $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{OH}) \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ .
15.  $\beta$ -hydroxyglutamic acid,  $\text{HOOC} \cdot \text{CH}(\text{NH}_2) \cdot \text{CH}(\text{OH}) \cdot \text{CH}_2 \cdot \text{COOH}$ .
16. Ornithine,  $\alpha$ - $\delta$ -diaminovaleric acid,  $\text{HOOC} \cdot \text{CH}(\text{NH}_2) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$ .
17. Histidine,  $\beta$ -iminazolyl- $\alpha$ -aminopropionic acid,



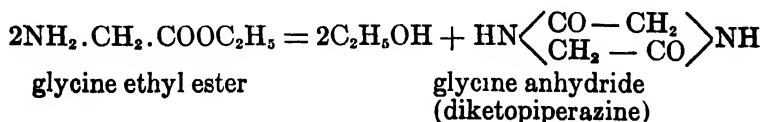
18. Proline,  $\alpha$ -pyrrolidinecarboxylic acid,



19. Oxypoline, *oxypyrrolidinecarboxylic acid*,  $\text{C}_5\text{H}_9\text{NO}_3$ .
20. Tryptophane,  $\beta$ -indole- $\alpha$ -aminopropionic acid,



The view that proteins are condensation products of amino acids was established by the classical work of Emil Fischer<sup>39</sup> on the synthesis of protein-like substances. He found that the esters of the amino acids are converted into anhydrides, upon heating, with elimination of two molecules of alcohol, thus



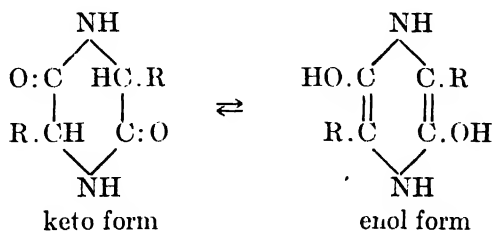


another 3, one 4, one 5 times, and 13 taken once each, there would be  $10^{27}$  isomers, even if there were no tautomerism of the peptide group and if the linking took place only in the simple way as with mono-amino-monocarboxylic acids. Holleman <sup>43</sup> points out that it is possible for each of the different kinds of living material to have its own individual protein and that the infinite variety of forms found in organic nature is partly the result of isomerism in the protein molecule.

The close resemblance of the more complex polypeptides to the natural proteins and to their first products of decomposition, the proteoses and peptones, and the fact that all proteins yield amino acids upon complete hydrolysis have established the view that the general structure of proteins is at least similar to that of the polypeptides, even though the straight chain, polypeptide theory of protein structure may have to be considerably modified.

Kossel <sup>47</sup> had already doubted that the various groups in the protein molecule are held together by primary valency forces and even Fischer <sup>39</sup> himself had pointed out the probable occurrence of piperazine rings. Troensegaard <sup>48</sup> has suggested that proteins are pyrrole derivatives. An interesting review of the modern work on protein structure has recently been published by Klarmann <sup>46</sup> and should be read for an account of the many theories that are being propounded.

Perhaps the most promising of the newer theories of protein structure is that they contain dioxopiperazine nuclei, a view advocated by a number of prominent workers, including Abderhalden and his collaborators.<sup>1-26</sup> They succeeded in preparing from simple amino acids a number of dioxopiperazines. These are also found in the degradation products of suitably hydrolyzed proteins. They exist in both the keto and enol forms and readily change from one form to the other under suitable chemical treatment, thus



where R represents an amino acid or polypeptide group. This tautomerism appears to play a very important rôle in the properties of some proteins.

Waldschmidt-Leitz and Schaeffer <sup>49</sup> showed that the very simple

dioxopiperazines, under physiological conditions, are not broken down by proteolytic enzymes or by the acidity or alkalinity of the digestive systems, whereas natural proteins are hydrolyzed. This seemed to indicate a weakness in the dioxopiperazine theory of protein structure and to strengthen the older view of amino acids held together by forces of primary valence. But Bergmann and Miekeley<sup>29</sup> found that certain isomeric forms of the dioxopiperazines can be built up into protein-like substances which can readily be hydrolyzed to amino acids without yielding the difficultly hydrolyzable dioxopiperazines.

Stiasny<sup>68</sup> has propounded the theory that proteins are built up in two ways: first the amino acids are linked together by forces of primary valence to form complex polypeptides or peptones and then these peptones are linked together by forces of secondary valence to form much larger aggregates. The protein is thus easily broken down to the peptone stage, but further hydrolysis of the peptones to amino acids is much more difficult. He gives this as the reason why pepsin breaks certain proteins down to the peptone stage only. Trypsin and erepsin break down peptones because they attack the  $\text{—CO—NH—}$  linkages. Stiasny's theory plays an important part in some theories of the mechanism of certain tannery processes.

Herzog and Gonell<sup>41</sup> examined collagen with the X-ray spectro-scope and concluded that it is much more simply constructed than had previously been supposed, being composed of a material having a molecular weight of about 700. This value is nearly that of the combining weight of 750 for collagen found by the writer and discussed in Volume II. Herzog's unit of 700 may correspond to Stiasny's peptone units making up the complete protein molecule. Herzog assumes the complexity of collagen as indicated by chemical analysis to be merely the result of impurities.

All that is definitely known of what might be regarded as the true molecular weights of the natural proteins is that they must be very large. In some jelly structures, it seems entirely possible that the polypeptide groups, or the dioxopiperazine structures, form continuous networks throughout the entire mass of jelly, there being no individual molecules in the orthodox sense of the term, just as we now know that a crystal of sodium chloride contains sodium and chlorine atoms, but no discrete molecules of sodium chloride.

The generally accepted methods of classifying proteins are based upon differences in solubility, speed of hydrolysis, and precipitability under definite conditions. But, since a small amount of foreign matter may alter these properties entirely for a given protein and because of

the difficulty of separating and purifying proteins, this system of classification is not wholly satisfactory, although it is, perhaps, the best available at the present time. The common names applied to proteins, such as keratin, albumin, etc., do not represent individual substances, but groups of closely related proteins whose quantitative separation is very difficult.

The most important classes of skin proteins, in the order of increasing importance to the tanner, are the mucins, albumins, globulins, melanins, keratins, elastins, the unnamed proteins of the grain surface, and the collagens. Except in the case of fur skins, the first five classes are of importance only because they must be removed from the skin prior to tanning, without injuring the remaining protein matter. In general, the albumins are the only skin proteins soluble in pure water. The globulins are soluble in dilute salt solutions and the mucins and melanins in dilute alkalis. The four remaining classes, which belong to the general group of proteins known as albuminoids, are insoluble, or difficultly soluble, in dilute solutions of acids, bases, or salts at room temperature, but all are dissolved and hydrolyzed by boiling solutions of concentrated acids or alkalis. The keratins are dissolved by strongly alkaline solutions before the remaining three classes are seriously attacked and the elastins are easily dissolved by trypsin before any injury is done to the collagen or grain surface. In boiling water, the collagen goes into solution as gelatin, leaving behind a residue of elastin and the proteins of the grain surface. The skin contains also a number of non-protein substances in the blood, lymph, and gland secretions, including salts, sugars, fatty materials, cholesterol, and soaps.

Bogue<sup>31</sup> has given a good review of the literature covering the chemical composition of skin, although the literature does not yet contain nearly all that the leather chemist would like to know of the composition of skin. Many measurements of the chemical constituents of skin are open to criticism because of the crudeness of the methods employed. Of the few measurements made in the field of leather chemistry, those of Rosenthal<sup>59</sup> and of McLaughlin and Theis<sup>50</sup> are deserving of special mention and will be discussed in connection with the description of the constituents of skin given below.

Rosenthal prepared his samples for analysis by drying the fresh skin in a vacuum oven at 55° to 60° C., cutting into small pieces, and then grinding to a coarse powder in a hashing machine. McLaughlin and Theis did not dry their skin samples, but they did shave away the hair, epidermis, and fatty layer so as to get the analysis of only

the corium, which represented about 80 per cent of the total thickness. This fact must be taken into consideration in attempting to compare their results with those of Rosenthal.

### **Albumins and Globulins.**

Albumins and globulins are found in the blood and lymph of the skin and also in the fluids of the muscles and nerves. They are characterized by the fact that they are coagulated from solution by the application of heat. The albumins are soluble in pure water or in dilute solutions of acids, bases and salts, but are precipitated by the addition of concentrated mineral acid or by saturating a weakly acid solution with salt. The percentages of different amino acids obtained from the albumins and globulins of horse serum are listed in Table I.

The globulins generally are insoluble in pure water at the neutral point, but dissolve in dilute neutral salt solutions, from which they can be precipitated by sufficient dilution or by saturating the solutions with salt, being most readily soluble in salt solutions of moderate concentration. They dissolve freely in dilute solutions of acids and alkalies. Like albumins, their solutions coagulate upon heating. Fibrinogen, an important constituent of the blood, is usually classed as a globulin, but differs from serum globulin in being precipitated from solution by a lesser concentration of neutral salt and of coagulating at a lower temperature. It tends to clot upon exposure to air, forming the insoluble protein fibrin, which action is favored by rise of temperature or agitation and is hindered by cooling or the addition of acids, alkalies, or concentrated salt solutions. The clotting action is supposed to be due to the action of an enzyme, thrombin, which is not a normal constituent of blood, but which is formed from the leucocytes and blood plates in the presence of calcium salts.

The elementary composition of serum albumin is given by Abderhalden as follows: carbon 53.08%, hydrogen 6.96%, nitrogen 15.93%, sulfur 1.9%, and oxygen 22.99%. For serum globulin he gives the following composition: carbon 52.71%, hydrogen 7.01%, nitrogen 15.85%, sulfur 1.11% and oxygen and other elements 23.32%. Analyses of this kind must be accepted with reservations. There are many kinds of albumins and globulins of apparently different elementary composition and the question of purity of any given sample may always be raised. However, even approximate values for nitrogen content are valuable in enabling one to get a rough estimate of the quantity of protein present in a mixture from the nitrogen value.



TABLE I.

PER CENT AMINO ACID OBTAINED FROM

Amino Acid	Horse Hair <sup>23</sup>	Keratin from Sheep Wool <sup>26</sup>	Horse Serum Albumin <sup>24</sup>	Horse Serum Globulin <sup>25</sup>	Elastin <sup>27</sup>	Collagen or Gelatin <sup>28, 29</sup>
Glycine .....	4.7	0.6	0.0	3.3	25.8	25.5
Alanine .....	1.5	4.4	2.7	2.2	6.6	8.7
Valine .....	0.9	2.8	...	...	1.0	1.0
Leucine .....	7.1	11.5	20.5	18.7	21.1	7.1
Isoleucine .....	...	...	...	...	...	0.0
Serine .....	0.6	0.1	0.6	...	...	0.4
Aspartic acid .....	0.3	2.3	3.1	2.5	...	3.5
Glutamic acid .....	3.7	12.9	7.7	8.5	0.8	5.8
Cystine .....	8.0	7.3	4.2	0.7	...	0.3
Phenylalanine .....	0.0	...	3.1	3.8	3.9	1.4
Tyrosine .....	3.2	2.9	2.1	2.5	0.3	0.0
Proline .....	3.4	4.4	1.0	...	1.7	9.5
Oxyproline .....	...	...	...	...	...	14.1
Oxyglutamic acid .....	...	...	...	...	...	0.0
Tryptophane .....	...	...	...	...	...	0.0
Histidine .....	0.6	...	3.4	2.8	...	0.9
Arginine .....	4.5	...	4.9	4.0	0.3	8.2
Lysine .....	1.1	...	13.2	2.2	...	5.9

Rosenthal<sup>59</sup> extracted the albumins and globulins from skin with a 10-per cent sodium chloride solution at 37° C. under toluene, combining three extractions, which were boiled with subsequent addition of a few drops of dilute acetic acid. The coagulum of albumins and globulins was washed with warm water, alcohol, and ether and weighed. For dog skin, he obtained, on the dry basis, 11.0% in the shoulder, 12.0% in the belly, and 9.6% in the butt. For calf skin, he found: shoulder 5.16%, belly 4.30%, and butt 4.14%. McLaughlin and Theis<sup>50</sup> extracted samples of corium with 5-per cent salt solution. Calculating their results to the dry basis, assuming an average of 63 per cent water, we get calf 5.1%, cow 1.0%, and steer 1.9%. Comparing the results with those of Rosenthal, it appears that practically all of the coagulable proteins are in the corium and that their amount is less in older animals.

### Mucins.

The mucins are conjugated proteins, of the group known as glycoproteins, containing both protein and carbohydrate groups in their molecules. They are insoluble in pure water, but, in faintly alkaline solution, give mucilaginous solutions which are precipitated by the addition of acid. Mucins are found in the secretions of the salivary glands and also associated with connective tissues.

No very sharp line of distinction can be drawn between the mucins

and the mucoids. Hammarsten <sup>40</sup> differentiates between them as follows: "The *true mucins* are characterized by the fact that their natural solutions, or solutions prepared by the aid of a trace of alkali, are mucilaginous, ropy, and give a precipitate with acetic acid which is insoluble in excess of acid or soluble only with great difficulty. The *mucoids* do not show these physical properties, and have other solubilities and precipitation properties."

Rosenthal <sup>59</sup> took his samples previously freed from coagulable proteins and extracted them with successive changes of half-saturated lime water. The combined extracts were neutralized to phenolphthalein and further acidified with 0.2-per cent hydrochloric acid solution. The precipitate was washed with 0.2-per cent acid, water, alcohol and ether, dried and weighed as mucoid material. His results follow: dog skin, shoulder 1.28%, belly 1.32%, and butt 2.01%; calf skin, shoulder 2.29%, belly 1.24%, and butt 4.81%. McLaughlin and Theis also extracted their samples with half-saturated lime water, but precipitated the mucoid with acetic acid. They found for calf skin 0.6%, cow hide 0.4%, and steer hide 0.4%. All figures are given on the dry basis.

Doubt is thrown on the above values for mucins or mucoid material by the fact that alkalis act upon the keratin of the epithelial cells yielding keratose, which is soluble in neutral or faintly alkaline solutions, but is precipitated practically quantitatively by acidifying to its isoelectric point of  $\text{pH} = 4.1$ , as given by Wilson and Merrill.<sup>78</sup> Thompson and Atkin <sup>67</sup> had previously shown that hair and wool are partially dissolved by lime liquors and that some of the matter dissolved is precipitated by rendering the solution slightly acid. It is significant also that McLaughlin and Theis obtained lower results than Rosenthal with skin from which the epidermis had been cut away. Even the samples used by McLaughlin and Theis probably contained appreciable quantities of epithelial cells. This is a matter of considerable practical importance, as will be shown in Chapter 10 on bating.

Cutter and Gies <sup>34</sup> determined the following elementary composition of mucoid material from tendon: carbon 47.47%, hydrogen 6.68%, nitrogen 12.58%, sulfur 2.20%, and oxygen 31.07%.

### Melanins.

The melanins are proteins of intense color, usually reddish-brown to black, constituting the pigment of the hair and epithelial cells. They

are insoluble in water and dilute acids, as a rule, but dissolve more or less readily in dilute alkalies. They may be extracted with boiling dilute alkali and precipitated by the addition of acid. They contain variable amounts of iron and sulfur in combination.

Abel and Davis<sup>27</sup> extracted the melanins from the skin of a negro and found: carbon 51.83%, hydrogen 3.86%, nitrogen 14.01%, sulfur 3.60%, oxygen and other elements 26.70%.

The development of the pigmenting materials of the skin is accelerated by exposure to strong sunlight. Prolonged exposure is followed by a rush of blood to the skin and the production of pigment which acts as a filter for ultraviolet rays, protecting the underlying tissues from the destructive action of these rays. The development of the pigment shows itself in the apparent darkening of the color of the skin, popularly known as tanning or sunburning. The coloring matter of the blood, hemoglobin, belongs to the class of conjugated proteins known as chromoproteins and, like the melanins, also contains iron and sulfur.

That the blood contains substances capable of reacting to produce deeply colored bodies is well appreciated by the tanners. Skins from which the blood has not been washed are likely to develop stains very difficult to remove later, unless special precautions are taken; these will be discussed in Chapter 7 in connection with methods for preserving skins for considerable periods before tanning.

### Keratins.

The chief constituent of the epidermal system, including the epidermis, hair, and epithelial cells of the glands, is the class of proteins known as keratin. The general method of preparing this material for examination is to boil with water the finely divided sample containing it, then to digest the residue with an acid pepsin solution followed by an alkaline trypsin solution and then to wash it thoroughly with water, alcohol, and finally with ether.

Keratin differs chemically from other classes of proteins in yielding a comparatively large amount of cystine, upon hydrolysis. In Table I are given the yields of amino acids obtained from keratins from horse hair and sheep wool. The differences shown by keratins from different sources are interesting, but each sample analyzed probably consisted of a mixture of different keratins more or less contaminated by other proteins.

Keratin prepared in the manner described above is naturally very

resistant to the action of dilute acids and alkalies, pepsin, trypsin, and boiling water, but it is dissolved by strong alkalies and by water heated to 150° C. under pressure. The method of preparation may be criticized on the ground that it does not include young keratin. On the other hand, it may be contended that the proteins of newly formed epithelial cells are not keratins at first, but are later converted into keratins. However, the changes in properties with age are so gradual as to make it almost impossible to draw any sharp line of demarcation. This is a good example of the difficulty of trying to classify proteins strictly according to properties. The cells of the Malpighian layer of the epidermis are readily attacked by trypsin and by solutions of ammonia, but become very much more resistant as they are pushed upward into the corneous layer.

In the *stratum granulosum* of the epidermis, the protoplasm of the epithelial cells has dried up and appears like granules inside of the cells. Walker<sup>70</sup> regards these granules as consisting of two substances, keratohyalin and eleidin, presumably stages in the transformation of the protoplasm into the wax and fatty material with which the cells of the corneous layer of the epidermis are loaded.

Rosenthal digested his skin samples, previously freed from coagulable proteins and mucoid material, first with trypsin and then with pepsin and called the insoluble residue keratin. In the dog skin he found, on the dry basis, shoulder 5.28%, belly 5.53%, and butt 6.41%; in calf skin, shoulder 36.15%, belly 25.73%, and butt 19.91%.

Regarding the elementary composition of keratins, van Laar<sup>81</sup> found for human hair: carbon 50.55%, hydrogen 6.36%, nitrogen 17.14%, sulfur 5.00%, and oxygen 20.95%. For wool, Schorer<sup>81</sup> found: carbon 50.65%, hydrogen 7.03%, nitrogen 17.71%, sulfur 4.61%, and oxygen 20.00%.

The tanner is much interested in differences between the properties of keratin and collagen in the process of unhairing. The cystine linkage, —S—S—, is so readily attacked by alkalies and sulfides as to render the keratin easily susceptible to hydrolysis by these reagents. Merrill<sup>52</sup> demonstrated that keratin, in contact with alkaline solutions at temperatures below 25° C., is much more readily hydrolyzed than collagen, whereas collagen is hydrolyzed the more readily in contact with acid solutions. He<sup>53</sup> also showed that sulfides react with keratin, forming compounds much more readily hydrolyzed by alkaline solutions than unaltered keratin, but that the sulfides have no such effect upon collagen. This work will be discussed more fully in Chapter 9.

### Elastins.

The yellow, elastic fibers interlacing the outer layers of the derma and enveloping the nerves and blood vessels are made up of a class of proteins called elastin. The tendons of the body have been the chief source of elastin used for study, in particular the *ligamentum nuchæ*, the tendon at the back of the head of the ox. F. L. Seymour-Jones<sup>60</sup> found that a piece of *ligamentum nuchæ* of about 1 square centimeter cross section gave on a testing machine an extension of 150 per cent before breaking, the strain being too small to measure; less than 5 lbs. He also found that the tendon was slowly digested by lime water, although the action may have been due to bacteria.

Elastin may be prepared for study by extracting this tendon with dilute sodium chloride solution, washing and then boiling it with water, then with a 1-per cent solution of potassium hydroxide, again with water, and then with acetic acid. The residue is then treated with cold 5-per cent solution of hydrochloric acid for 24 hours, thoroughly washed with water, boiled again with water, and then washed with alcohol and ether and dried. It then has a yellowish-white appearance. It is not dissolved by boiling water or by acids and alkalis in the cold, but is easily dissolved by concentrated mineral acids upon heating. The yields of the different amino acids from a sample of elastin are given in Table I.

In elastin from *ligamentum nuchæ*, Chittenden and Hart<sup>33</sup> found carbon 54.24%, hydrogen 7.27%, nitrogen 16.70%, and oxygen 21.79%.

It is, of course, not safe to assume that elastin from skin has exactly the same properties as that from other parts of the body, but the difficulty of isolating some of the skin proteins for study has made it desirable to investigate proteins of the same general classes from parts of the body where they are more easily available, if only to get a suggestion of the properties of the skin proteins. Actually we do find that the elastin of skin behaves much like that from the *ligamentum nuchæ*, being resistant to boiling water and to cold solutions of acids and alkalis. In glue manufacture, much of the elastin remains in the scutch or residue left after boiling the skin in water.

The elastin fibers of skin are attacked only very slowly by dilute solutions of acids or alkalis, but are easily dissolved by neutral trypsin solutions. A detailed account of the action of trypsin upon elastin will be given in Chapter 10. Wilson and Daub<sup>72</sup> found that saturated lime water at 20° C. begins to attack the elastin fibers of

calf skin appreciably only after 3 weeks' contact and completely destroys them in about 5 weeks.

In an unpublished work carried on in the author's laboratories, Merrill and Daub studied the action of sulfides on the elastin fibers of calf skin. They put strips of calf skin into saturated lime water at 20° C. containing different amounts of calcium sulfhydrate and noted the destruction of elastin by examining sections under the microscope, from time to time. With either 0.01 or 0.09 mole per liter of  $\text{Ca}(\text{SH})_2$  the rate of digestion was exactly the same as with pure lime water, from which it was concluded that the sulfide was without action on the elastin.

Rosenthal's method of estimating the amount of elastin in his skin samples was to take them after extraction of coagulable proteins and mucoid material and to subject them to an alkaline tryptic digestion under toluene at 37° C. After 24 hours the solution was decanted off and the digestion was continued for 96 hours with fresh pancreatin. Nitrogen in the combined filtrates was determined and multiplied by the factor 6 for elastin, making due allowance for nitrogen derived from the enzyme. His results were, for dogskin, shoulder 2.41%, belly 5.49%, and butt 2.05%; and for calfskin, shoulder 16.74%, belly 19.43%, and butt 12.31%, all figures being on the dry basis. In their analysis of only the corium of the skin, McLaughlin and Theis found for calfskin 0.05%, cow hide 0.27%, and steer hide 0.92%, on the dry basis.

The very high results obtained by Rosenthal may be attributed to the probable inclusion of derivatives of keratin. A discussion of the action of trypsin on keratose is given in Chapter 10. The skins used by McLaughlin and Theis in their measurements had previously been freed from epidermis and hair. An examination of the photomicrographs in Chapter 10 will permit a very crude, but direct, estimation of the proportion of elastin fibers to the rest of the connective tissues.

### Reticulin.

Lloyd<sup>43</sup> states that Miss Madge Kaye has isolated a tissue from skin that is known as reticulin. Photomicrographs of skin sections showing unswollen bands of reticulin among collagen fibers swollen by acid are given in a paper by Kaye and Lloyd.<sup>44</sup> According to Lloyd, the reticulin forms an exceedingly fine filamentous network penetrating the cellular organs, connective tissue and skin, and apparently forming a framework for a great deal of the cellular tissue in the

animal body. Reticulin is remarkable for its great resistance to the action of chemical reagents, being unaffected, structurally at least, by boiling water or boiling dilute acids and alkalies and able to withstand the action of cold, concentrated solutions of acids and alkalies for many hours. It is not attacked in its fresh condition by pancreatic trypsin, but is rapidly attacked by pepsin. The relative amount of reticulin in the skin must be very small.

### Proteins of the Grain Surface.

The proteins of the grain surface are remarkably resistant to most of the ordinary chemical reagents. The thin fibers of this surface are not dissolved by solutions of caustic alkalies sufficiently strong to destroy the collagen fibers, epidermis and hair. In boiling water, they evidently undergo some change in composition, but remain undissolved in the form of a thin sheet while the collagen passes into solution as gelatin. They are apparently unaffected by trypsin solutions strong enough to dissolve all of the elastin fibers beneath them. But in contact with water having a pH value between 6.5 and 8.0, they are easily attacked and liquefied by putrefactive bacteria, although this action can be checked by the addition of a sufficient amount of acid, alkali, or salt. This will be discussed more fully in Chapter 7.

These fibers represent only a very small proportion of the skin by weight, but they are of great importance because they form the grain surface of finished leather, giving it its characteristic appearance. Their position in the grain surface is shown in Plate 19 of Chapter 2. In tanning and dyeing, they take a color different from that assumed by the collagen fibers, which is noticeable when leather is cut. Any damage to the grain surface reduces the selling value of the leather materially.

### Collagen.

Collagen is the most abundant protein of the skin and the one of greatest importance to the tanner, since it is the basis of leather. It constitutes the bulk of the substance of the white fibers of the connective tissues of the derma.

Collagen can be prepared for study from fresh skin by removing the other constituents. The adipose tissue is carefully cut away and the skin thoroughly washed. It is then extracted with several changes of 10-per cent sodium chloride solution, in a closed jar set in a tumbling machine, or agitator, in order to remove the soluble protein matter. It is then put back into the same jar with a one-tenth-per

collagen was studied by Thomas and Foster.<sup>56</sup> They exposed dry hide powder in an open dish to the rays of a Cooper-Hewitt mercury arc quartz lamp (about 1000 candlepower) for a period of several weeks. The contents of the dish were stirred frequently, since the rays can penetrate but a very thin layer of hide powder. The ozone formed through the action of the ultraviolet light was continuously pumped off at the top of the lamp house. The hide powder assumed a bright canary yellow color and the same action was observed with a sample of hide powder enclosed in a quartz bottle in an atmosphere of nitrogen.

Analysis of the irradiated hide powder showed  $17.96 \pm 0.05$  per cent nitrogen, on the absolutely dry basis, a trifle more than that accepted for ordinary hide powder (17.82 per cent). When placed in water, irradiated hide powder gave evidence of having undergone a deep-seated change chemically, in that a yellow solution arose containing something precipitable by tannin. An amount equal to two grams of dry powder was drummed with 200 cc. water for six hours at room temperature. Analysis of the solution showed that 27.8 per cent of the irradiated powder had dissolved.

They also found that the capacity of the hide powder to combine with hemlock tannin was very greatly reduced as a result of the effects produced by exposure to ultraviolet light. When the irradiated hide powder was submitted to quinone tannage, it combined with practically the same amount of quinone as untreated hide powder, indicating a difference in nature between the two kinds of tannage. No satisfactory explanation has yet been offered for the mechanism of the action of ultraviolet light upon collagen.

It is interesting here to note that Meunier and Rey<sup>54</sup> found that exposure of wool to ultraviolet light made the sulfur groups more reactive. After exposure, wool reacts acid to methyl red and it also reacts more rapidly with quinone and less rapidly with alloxan.

After removing the coagulable proteins, mucoid material, and elastin from his skin samples, Rosenthal<sup>59</sup> digested them with pepsin in a 0.2-per cent hydrochloric acid solution under toluene at 37° C. for 24 hours and then for 96 hours with fresh solution, filtering off the solution after each digestion. The nitrogen value of the combined filtrates was calculated to collagen by multiplying by 5.58. On the dry basis, he found for dog skin: shoulder 32.74%, belly 21.13%, and butt 19.59%; for calf skin: shoulder 39.66%, belly 51.46%, and butt 58.83%. McLaughlin and Theis<sup>60</sup> found for their samples previously freed from epidermal layer and hair: steer hide 85.1%, cow hide 87.2%, and calf skin 84.0%, on the dry basis.

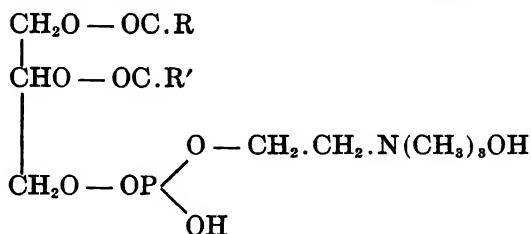


The chemistry of collagen and gelatin forms so large a portion of the chemistry of leather manufacture that further treatment must be reserved for the appropriate chapters.

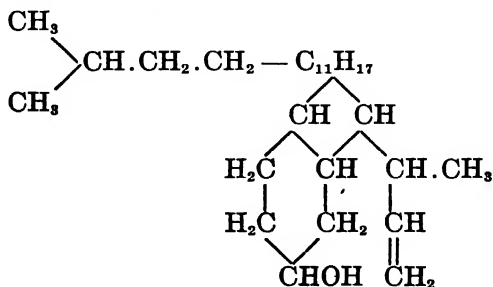
### Fatty Constituents.

Except for the recent work of McLaughlin and Theis,<sup>51, 54</sup> very few studies seem to have been made of the fatty constituents of animal skin used for making leather. The terms lipoids and lipins are sometimes used to include, not only true fats, but also the other constituents of the animal body which are soluble in organic solvents, such as waxes and fatty acids combined with protein. In skin, lipins occur in the cells of the sebaceous glands, adipose tissues, and fatty cells of the corium and also in the blood and lymph.

Besides the true fats, the important lipins of the skin are the lecithins and cholesterol. The lecithins are fats in which one fatty acid radical has been replaced by a compound of phosphoric acid and choline (trimethoxyethyl ammonium hydroxide). thus:



where R and R' represent the fatty acid radicals. Cholesterols are secondary alcohols belonging to the group of terpenes. Windaus<sup>57</sup> gives the following formula



The structure of the radical  $\text{C}_{11}\text{H}_{17}$  is still unknown.

Lecithin readily forms an emulsion in water without the assistance of other materials. Cholesterol shows a very much lower attraction

for water, but its emulsions may be stabilized by the addition of lecithin, which acts as a protective colloid. Both are soluble in alcohol and ether, and both present a waxy appearance. The melting point of purified cholesterol is 149° C.

McLaughlin and Theis<sup>50</sup> extracted skin, with epidermis and hair previously split away, with acetone, absolute alcohol and finally with ether and obtained the following values for total fatty matter: steer hide 0.76%; cow hide 0.13%, and calf skin 0.45%. These values are calculated on the fresh weights of the skins and must be multiplied by about 3 to get the values on the dry basis. They also showed that the fatty materials are concentrated in the outer layers of the skin, being relatively scarce in the middle layer. After extracting the steer hide as indicated above, they continued the extraction with acetone and alcohol containing varying proportions of acetic acid. With increasing concentration of acid, increasing amounts of fatty material were extracted. Where the solvents contained 10 per cent of acetic acid, more than three times as much material was extracted from steer corium as when no acid was present. Apparently much of the fatty material is so associated with the protein materials that it cannot be extracted by organic solvents until the protein materials have been hydrolyzed. This hydrolysis is also brought about by bacterial action and is accompanied by an increase in acid value of the extracted fats. When skins are allowed to stand around after flaying, there is also an increase in per cent of oxidized fatty acids. Curing with sodium chloride retards the action.

McLaughlin and Theis<sup>50</sup> examined the fat from steer, calf, and goat corium and obtained the results shown in Table II.

TABLE II.

	FAT EXTRACTED FROM CORIUM OF			Beef Tallow
	Steer	Calf	Goat	
Iodine value .....	44-48	15.9	25.5	38-46
Saponification value .....	193.2	199.2	177.0	193-200
Refractive index at 25° C .....	1.46	1.49	1.46	1.45
Per cent unsaponifiable .....	3.35	12.68		
Per cent nitrogen .....	0.68			
Per cent phosphorus .....	0.25			
Per cent mixed fatty acids .....	77-83			
Solid fatty acids (% of total)....	25-29			
Liquid fatty acids (% of total)....	75-71			
Iodine value mixed acids .....	47-55			41.3
Iodine value liquid acids .....	80			92.4
Refractive index solid acids .....	1.44			
Refractive index liquid acids .....	1.46			

## Mineral Constituents.

Besides the fatty matters, the blood and lymph in skin contain also a number of other non-protein substances, including sugars and salts, particularly the phosphates, carbonates, sulfates, and chlorides of sodium, potassium, magnesium, and calcium. Iron and sulfur are

TABLE III  
MINERAL CONSTITUENTS OF SKIN CORIUM.  
(Percentages Figured on Fresh Weight of Corium)

	Steer	Cow	Calf	Bull	Heifer	Goat *
Ash .....	0.4530	0.3630	0.4950	0.4920	.....	2.85
SiO <sub>2</sub> .....	.....	0.0037	0.0048	.....	.....	.....
Fe <sub>2</sub> O <sub>3</sub> + Al <sub>2</sub> O <sub>3</sub> .....	0.0107	0.0190	0.0134	0.0124	0.0194	0.5650
CaO .....	0.0101	0.0038	0.0095	0.0124	0.0038	0.0190
MgO .....	0.0032	0.0036	0.0073	0.0039	0.0034	0.0253
NaCl .....	0.4450	0.3530	0.4430	0.4825	0.4410	.....
Cl .....	0.2730	0.2130	0.2690	0.2930	0.2670	.....
SO <sub>3</sub> .....	0.0702	0.0614	0.0952	0.0689	0.0685	1.0480
P <sub>2</sub> O <sub>5</sub> .....	0.0318	0.0262	0.0829	0.0334	0.0181	0.0956
Ratio MgO: CaO .....	1:3.20	1:0.14	1:0.11	1:0.37	1:0.18	.....
Ratio P <sub>2</sub> O <sub>5</sub> : CaO .....	1:0.32	1:0.14	1:0.11	1:0.37	1:0.18	.....

\* Whole sun-dried skin; previous history unknown.

TABLE IV.  
MINERAL CONSTITUENTS OF ANIMAL SKINS FREED FROM HAIR AND FLESH  
(DRY BASIS).

No. Skins Analyzed	Animal	Milligrams Per 100 Grams Dry Skin			
			Ca	Mg	Na K
10	man	max.	59	38	408 339
		min.	34	20	298 168
		av.	46	30	360 239
10	dog	max.	58	37	250 395
		min.	31	21	155 158
		av.	43	27	201 238
18	rabbit	max.	86	52	243 188
		min.	51	17	116 102
		av.	74	35	181 148

found in the hemoglobin of the blood. Table III gives the mineral contents of a number of fresh skins, as determined by McLaughlin and Theis.<sup>50</sup> The skins have been freed from the epidermal and hairy layers and the figures are given on the fresh weight and should be multiplied by three to get the approximate per cent on the dry basis. The exception to this is the sun-dried goat skin, on which the figures apparently represent per cent of the dry weight.

Table IV gives the calcium, magnesium, sodium, and potassium contents of human, dog and rabbit skins as determined by Brown.<sup>52</sup> The hair and adhering flesh were removed as completely as possible

## Chapter 4.

### Measurements of Acidity and Alkalinity.

Rigid control of the acidity, or alkalinity, of tannery liquors is of vital importance in producing leather of uniform quality, for relatively small variations in acidity of the liquors often result in wide variations in properties of the finished leather. By juggling the methods of operation until a nearly uniform product was obtained and then rigidly adhering to a developed process, tanners long ago perfected means for keeping hydrogen-ion concentrations reasonably well under control, although without any appreciation of the physico-chemical laws governing the operations. When the acidity of any liquor got beyond the control of the operator and rose or fell to an abnormal value, the result was apt to be disastrous unless the tanner had learned from similar experiences how to save the stock from further damage.

The development of the hydrogen electrode has made possible the measurement of hydrogen-ion concentration, or active acidity or alkalinity, of tannery liquors and this has brought about a tremendous advance in the science of leather making. Since the appearance of the first edition of this book, the use of the hydrogen electrode in tanning practice has greatly increased and a demand has arisen for a detailed account of hydrogen-ion measurements as applied to tannery liquors. This demand will be met in this chapter by a description of the routine methods developed in the author's laboratories during the past twelve years. For an account of the fundamental principles of concentration cells and of the many types of cells in general use, reference should be made to the excellent books devoted entirely to this subject, which are readily available, such as those of Clark<sup>18</sup> and Michaelis.<sup>20</sup>

When a metal is dipped into a solution of one of its salts, an electrical potential difference is established between the metal and the solution due to differences in the tendencies for metal to pass into solution as ions and for ions to leave the solution and become deposited on the metal. The magnitude and sign of this potential difference

is determined by the nature of the metal and the concentration of the ions of the metal in the solution. In the familiar Daniell cell, an electrode of copper is dipped into a solution of copper sulfate and an electrode of zinc is dipped into a solution of zinc sulfate. The zinc atoms have a great tendency to pass into solution as zinc ions, leaving their valence electrons on the zinc electrode, charging it negatively. The copper ions have a tendency to leave the solution, leaving their electrons on the sulfate ions in the solution and charging the copper electrode positively. When contact is made between the two solutions, using a porous plate to prevent the zinc sulfate and copper sulfate from mixing, the system becomes an electric battery with the zinc electrode the negative pole and the copper electrode the positive pole and the voltage of the battery is the potential difference between the two electrodes. If the voltage of the battery and the potential at one of the electrodes are known, the potential at the other electrode can be calculated by difference.

If a battery is so constructed that the potential at one electrode is kept constant at known value, then the voltage of the battery will vary as the potential at the other electrode. And, if the only variable factor operating at the second electrode is concentration of the active ion in the solution, the voltage of the battery will be a measure of this concentration. This principle is used in many kinds of measurements of ion concentrations, including that of hydrogen-ion concentration.

In measuring hydrogen-ion concentration, the electrode of fixed potential is the so-called calomel electrode, consisting of mercury in contact with a saturated solution of mercurous chloride and potassium chloride. The variable electrode is a platinum wire whose surface is saturated with hydrogen gas, making it act towards the solution like an electrode of hydrogen, the platinum itself being practically inert. With increase in hydrogen-ion concentration in the solution, there is a corresponding increase in tendency for hydrogen ions to pass from the solution to the hydrogen electrode, increasing its positive electrical charge. Where hydrogen-ion concentration is the only variable, it can be measured by the variation in voltage of the battery as a whole.

The literature cited above should be consulted for the theoretical treatment of concentration cells and the methods employed in deriving the equations relating the ion concentration at the variable electrode to the voltage of the complete battery. The working rules given below, however, will enable anyone of ordinary laboratory experience



librium more rapidly, which is desirable in titrating and in liquors that easily poison the electrode. Dozens of these electrodes should be kept under water ready for use, and it is desirable to have a number in distilled water with hydrogen bubbling over them, where they may be needed on short notice for liquors which poison the electrode easily. They are readily cleaned, before replatinizing, by dipping into hot aqua regia for a moment.

Vessel *C* is the calomel cell. At point *D* the tube is sealed off with a platinum wire making electrical contact between the separated portions. The side arm *E* is filled with mercury, into which is inserted one of the wires from the potentiometer. The bulb of the cell is filled with mercury covered with a layer of mercury-mercurous chloride paste, and the cell is kept filled with a saturated solution of potassium chloride and mercurous chloride, which enters the cell from a large reservoir through the side arm *F*, the flow being regulated by stopcock *G*. When the cell is being flushed with the chloride solution, the overflow passes out through the capillary side arm *H*. The solution whose hydrogen-ion concentration is to be measured is poured into dish *I*.

The deep U in the capillary tube prevents contamination of the calomel cell by the tannery liquor, which usually has a lower specific gravity than the solution in the cell. Stopcock *G* permits flushing the capillary tube after each determination. One of these cells may be used daily for many months without other cleaning.

### Platinizing the Electrodes.

Before using, the exposed portion of the platinum wire in the hydrogen electrode must be coated with a thin deposit of platinum black. Two electrodes are dipped into a solution made by dissolving 3 grams of platinic chloride in 100 cc. of half-normal hydrochloric acid solution so that the platinum electrodes are entirely immersed and about 2 cm. apart. Through the mercury in the glass tubes, the electrodes are connected to a 4-volt storage battery. The electrode acting as the cathode will gradually become covered with a deposit of platinum black, which will increase in thickness until the current is stopped. A little skill, gained through experience, is required to judge the most desirable thickness of deposit. If it is too thin, the electrode will become poisoned too easily in use; if too thick, it will be sluggish and will not reach equilibrium quickly. Under the conditions described, about 5 minutes are required to get a deposit of the right thickness, although the time will vary with the current flow and with

the condition of the platinum surface. It is, of course, obvious that the platinic chloride solution must be strengthened or replaced from time to time, as indicated by its behavior when platinizing. It is still possible to platinize the electrodes in a solution reduced to one-third of its original strength, although a much longer time of platinizing is then required. A few drops of dilute hydrochloric acid added to the platinizing solution sometimes assists in the formation of a better deposit. Because an absolutely pure solution of platinic chloride will give a bright deposit of platinum, some chemists add about 20 mg. of lead acetate to 100 cc. of the solution to ensure formation of a black coating. However, the author has never found this necessary with the ordinary c.p. platinic chloride.

After an electrode has been platinized, it is made the cathode in a cell in which a current is passed through dilute sulfuric acid solution for several minutes. It is then set in its holder immersed in distilled water and hydrogen is bubbled over it for several minutes, after which it is kept under distilled water until required for use. It must never be allowed to dry. In tannery routine work, it is desirable to keep a dozen or more electrodes set in test tubes of distilled water ready for immediate use.

When an electrode has become poisoned through use, it is cleaned by dipping it into boiling aqua regia. When the metal again appears bright, it is rinsed with distilled water and replatinized.

### Preparing the Calomel Cell.

The calomel electrode vessel must first be thoroughly cleaned with a hot sulfuric acid-sodium dichromate solution and then with distilled water. About 15 grams of highly purified mercury is put into vessel *C* so that contact is made with the platinum wire. About 5 grams of mercury and 5 grams of very pure mercurous chloride are mixed dry by shaking together and placed on top of the mercury in vessel *C*. In a reservoir bottle of 'about 2 liters' capacity is kept a saturated solution of potassium chloride and a maintained excess of potassium chloride crystals. This reservoir is connected to vessel *C* through the side arm *F* and the vessel and side arm *H* are filled with the solution.

### Making the Determination.

The apparatus is set up as shown in Figure 2. The unknown solution is poured into dish *I*. If the hydrogen is taken from a tank of ordinary commercial hydrogen, it is necessary to clean it. This is



done by passing it through a series of gas washing bottles, the first containing a saturated solution of mercuric chloride, the second a strong solution of potassium permanganate and sodium hydroxide, and the third an alkaline solution of pyrogallol. Where the tank hydrogen is pure enough, the washings with mercuric chloride and permanganate are unnecessary. It is then passed through a tower of cotton fibers and then through the electrode vessel so that it bubbles over the platinized electrode.

Wires, as indicated in Figure 2, lead from the electrodes to a Leeds and Northrup portable potentiometer with self-contained Weston cell, dry battery, and galvanometer and the voltage of the system is read. If the voltage continues to rise over a period of time, it indicates that equilibrium has not been established. A falling or fluctuating voltage results from an electrode which is being poisoned by the solution. When the voltage remains constant for two or three minutes, the reading is taken and the hydrogen-ion concentration calculated from the formula

$$-\log[H^+] = \frac{V - 0.2450}{0.0001983T}$$

where  $V$  is the measured voltage and  $T$  the absolute temperature ( $273^\circ$  plus temp.  $^\circ\text{C}.$ ). The brackets indicate concentration of hydrogen ion in moles per liter. For temperatures far removed from  $25^\circ\text{C}.$ , it is necessary to increase the value 0.2450 by 0.0002 for each degree C. rise above  $25^\circ$  and to decrease it by 0.0002 for each degree fall below  $25^\circ$ . Fales and Mudge<sup>6</sup> found the temperature coefficient of the saturated potassium chloride calomel cell to be 0.0002 volt per degree C. from  $5^\circ$  to  $60^\circ$ .

A battery of these electrodes actually in use in a tannery laboratory is shown in Volume II. The hydrogen tank is below the table and the gas washing bottles and potassium chloride reservoirs are on the roofing. There are 8 sets of electrodes, each connected to a double-pole, double-throw switch. A throw in one direction connects the set to a recording potentiometer and a throw in the other direction to a more accurate potentiometer for the final reading. In running 8 determinations simultaneously, the operator first connects no. 1 with the recording potentiometer. When the line being drawn reaches the vertical position, he switches to the more accurate potentiometer and makes the final reading. As soon as no. 1 is disconnected from the recording potentiometer, no. 2 is connected so that its line is being

drawn while the determination is being completed on no. 1. In this way, much time is saved in routine work.

As one example of the method of calculation, the result obtained with a pickle liquor will be given. The temperature was 25° C., which is 298° on the absolute scale. The value of  $0.0001983T$  is thus 0.0591. The measured voltage was 0.3337. Subtracting 0.2450, we get 0.0887. Dividing this by 0.0591, we get 1.50, the value of  $-\log[H^+]$ . The number whose logarithm is  $-1.50$  is 0.0316. This is the number of

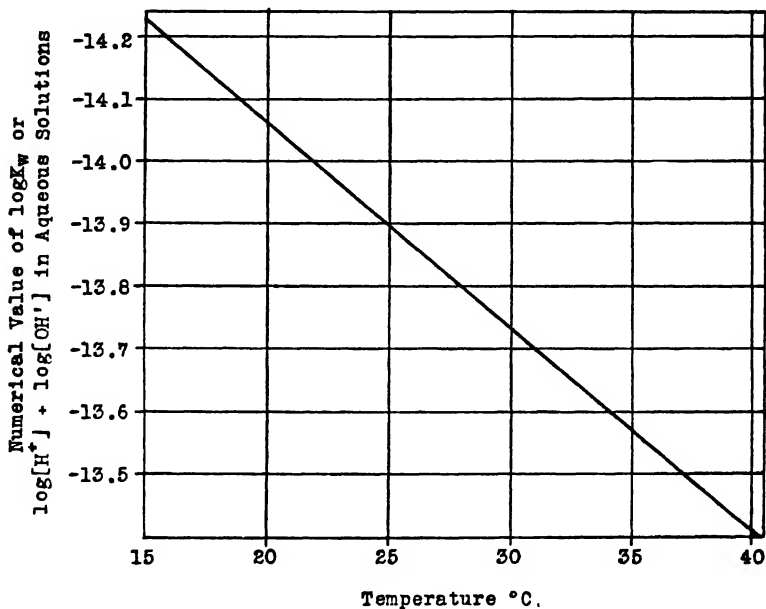


FIG. 3.—Showing how the relation of  $[H^+]$  to  $[OH']$  in aqueous solutions varies with temperature. The values indicate the degree of ionization of water as a function of temperature.

moles of hydrogen ion per liter, or the normality. Similarly a lime liquor gave a voltage reading of 0.9738 at 21° C. Since this is 4 degrees lower than 25°, we must subtract 0.0008 from 0.2450. Taking 0.2442 from 0.9738, we get 0.7296. This divided by 0.0583 is 12.51, the value of  $-\log[H^+]$ . Since we are dealing with an alkaline solution, it is more convenient to have the value in terms of hydroxide-ion concentration. In aqueous solutions at 21° C. the hydrogen- and hydroxide-ion concentrations are so related that  $-\log[OH'] = 14.03 + \log[H^+]$ . Therefore the value of  $-\log[OH']$  is  $14.03 - 12.51$ , or 1.52. The number whose logarithm is  $-1.52$  is 0.0302. This is the number of moles of hydroxide ion per liter.

Calculation of the hydroxide-ion concentration of an aqueous solution from a knowledge of the hydrogen-ion concentration is based upon the principle of constancy of the product  $[H^+] \times [OH']$  in any aqueous solution at constant temperature. This product is usually written  $[H^+] \times [OH'] = K_w$ . The value of  $K_w$  is usually taken as  $10^{-14}$  for all ordinary temperatures, or  $\log K_w = -14$ . Actually  $\log K_w$  varies with temperature as indicated in Fig. 3, which can be used to advantage when calculating the hydroxide-ion concentrations of alkaline solution from hydrogen-ion measurements. At the true neutral point,  $\log[H^+] = \log[OH']$ . But  $\log[H^+] + \log[OH'] = \log K_w$ . Therefore, at the neutral point  $\log[H^+] = \log K_w/2$ , which can be read from Fig. 3.

### pH Value.

Instead of reducing the value of the expression  $-\log[H^+]$  to actual hydrogen- or hydroxide-ion concentration, it is preferable to use the value itself, because of the enormous range of hydrogen-ion concentrations found in the tannery. A more convenient term is pH value, which means  $-\log[H^+]$ .

The term pH value is now widely used throughout the industrial world, even by men with no chemical training at all. Men in charge of industrial processes accept pH values as a standard scale of acidity and alkalinity, as they do a thermometer scale for temperature, without caring about its mechanism. They learn, for example, that a certain type of liquor works best at a pH value of 5.5. When the analyst reports a value for this liquor of 6.5, they immediately appreciate that the addition of acid is necessary to bring the liquor back to 5.5. They have no difficulty in understanding that  $pH = 7$  represents a neutral solution, that values increasing from 7 indicate increasing alkalinity and values decreasing from 7 indicate increasing acidity.

For the leather chemist, the adoption of the pH scale has the advantage of eliminating the use of negative values and of making his system of record more desirable for use by men not trained to use logarithms, negative values, and theories of ionization.

It is surprising how quickly unschooled men learn to make pH measurements. Setting up the apparatus and measuring the voltage of the system are simple mechanical operations. Since, for any given temperature, the pH value of the unknown liquor is a straight-line function of the measured voltage, it is possible to prepare a series of graphs, one for each appreciable interval of temperature, from which

pH values can be read off directly as functions of voltage by any good workman. Fig. 4 shows a typical chart of wide range; for special

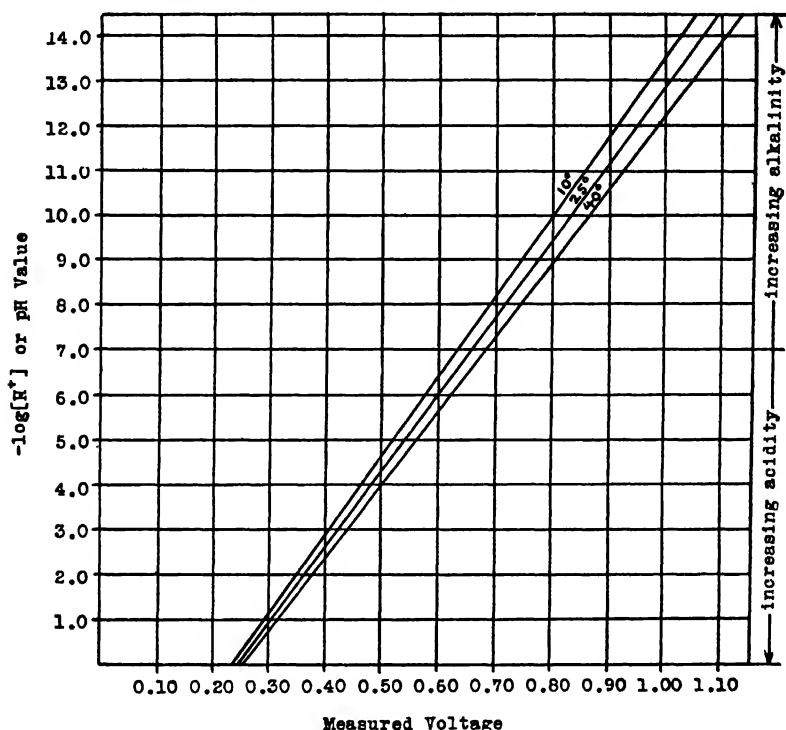


FIG. 4.—Difference of potential between hydrogen electrode in contact with solutions of different pH values and saturated potassium chloride calomel electrode at 10°, 25° and 40° C.

work, it is desirable to have much more sensitive charts covering only the necessary working range. These charts are great time savers, even for trained chemists.

### Total Acidity.

While the chief use of the hydrogen electrode in the tannery is to measure the active acidity or alkalinity of liquors in terms of pH value, it is of great value also in indicating the total amounts and nature of the acids or alkalies present and in showing exactly how much of a given reagent to add to change the pH value in a given manner. This is done by measuring the change of pH value of a liquor while titrating it with a standard solution of the reagent. The recording potentiometer is very useful in this type of work. The

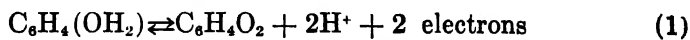
titration curves obtained often tell very interesting stories about the liquor. Two liquors may show exactly the same pH value, say 2.5, indicating equal activities of acid, but the first may require very little alkali to raise the pH value to 7, while the second may require several times as much. This would show that the first liquor contained a small amount of a relatively strong acid and the second a larger amount of substances of weakly acidic nature, which would act as buffers against the introduction of alkalies, often a very important factor in tanning.

### The Quinhydrone Electrode.

In the electrometric determination of pH value in acid solutions, it is possible to replace the hydrogen electrode by an electrode in which benzoquinhydrone is used in place of hydrogen. Certain materials, such as oxidizing agents, interfere with the proper operation of the hydrogen electrode and are said to poison it, making the measurement of pH value impossible. For a number of such liquors the quinhydrone electrode has been found to give satisfactory measurements of pH value. Possible uses of this electrode for tan liquors have been suggested and discussed by Hugonin.<sup>11</sup> Parker<sup>25</sup> has described a continuous-flow type of quinhydrone electrode which the author found to work very satisfactorily for the measurement of pH value of the filtrate from acidified sewage sludge, which was impossible with the hydrogen electrode because of a poisoning effect.

In making the measurement of pH value with the quinhydrone electrode, a few crystals of quinhydrone are dissolved in the solution to be measured, a platinum electrode is inserted and a calomel half cell is connected as in the case of the hydrogen electrode. The voltage of the system is a measure of the oxidation-reduction potential at the platinum surface. The electrode may be made of bright platinum, or better, of gold plated platinum.

The theory of the method is based upon the observations of Haber and Russ<sup>9</sup> and of Granger and Nelson,<sup>8</sup> who have shown that benzoquinhydrone in aqueous solution is highly dissociated into its equimolecular components quinone and hydroquinone, which are in equilibrium with each other according to the following electrochemical equation:



Since the reaction is strictly reversible, Peter's form of the van't Hoff isotherm is applicable and we have

$$\pi_m = \pi_o + \frac{0.000198T}{2} \log \frac{[C_6H_4O_2]}{[C_6H_4(OH)_2]} + 0.000198T \log [H^+]. \quad (2)$$

In this equation  $\pi_m$  is the measured electrode potential and  $\pi_o$  is the normal potential of the system; namely + 0.6990 volts on the hydrogen-electrode scale. Thus the electrode potential is a linear function of the logarithm of the hydrogen-ion concentration, or pH value, as long as the reaction remains uncomplicated by side reactions.

Biilmann<sup>2-4</sup> was the first to advocate the method as a means of measuring pH value. He pointed out, since the benzoquinhydrone yields equimolar concentrations of quinone and hydroquinone, that the term involving the concentration ratio under such conditions vanishes and we have

$$-\log[H^+] = \text{pH} = \frac{\pi_o - \pi_m}{0.000198T} \quad (3)$$

an equation identical with that given above for the hydrogen electrode, except for the value of  $\pi_o$ .

LaMer and Parsons<sup>17</sup> found the value of  $\pi_o$  to be + 0.6990 volt at 25° C., using the saturated KCl calomel cell as the opposing electrode. The same value was found by Biilmann. Consequently, pH values of solutions at 25° C. may be calculated from the following equation

$$\text{pH} = \frac{0.6990 - V}{0.059} \quad (4)$$

where V is  $\pi_m$  or the voltage measured between the quinhydrone electrode and the saturated KCl calomel cell.

The validity of this equation depends upon the presence of exactly equal concentrations of quinone and hydroquinone; this is the basis for the elimination of the term involving the logarithm of the ratio of concentrations in equation (2). The simplified equation is no longer valid when substances are present which either oxidize hydroquinone or reduce quinone. Oxidizing agents which interfere are ferric, permanganate, and dichromate ions and others of similar oxidizing power. Among the reducing agents which interfere are iodide, titanous, and chromous ions. Since hydroquinone solutions are easily oxidized by oxygen from the air at pH values higher than 8, it is not possible to use the quinhydrone electrode except in neutral or acid solutions with any degree of satisfaction. High concentrations of salts also interfere.

As yet all interfering substances are not known, so that the applicability of the quinhydrone electrode to any particular liquor can only

be ascertained by experience. The values obtained with this electrode for tan liquors should not be accepted until it has been demonstrated that they would be the same as those obtainable with the hydrogen electrode. A check might be obtained in one tannery with one type of tan liquor and not in another with tanning materials of different origin or treatment. The presence of any pair of organic compounds which oxidize or reduce from one to the other would constitute a quinhydrone-type of electrode with its own characteristic potential which might be quite different from that of quinhydrone.

### Indicators.

There are many colored substances soluble in water that undergo a change in color with a change in pH value of the solution, making it possible to use the color of the solution as a measure of its pH value. These indicators find a place in the tannery in controlling the pH values of bate liquors and of waters of low conductivity. Phenol-sulfonphthalein, or phenol red, is yellow at pH values less than 6.8 and red at pH values higher than 8.4. If a series of solutions is prepared, ranging in pH value from 6.8 to 8.4, in intervals of 0.1, and to a small test tube of each is added a fixed amount of phenol red solution, a series of colors will be obtained, ranging from yellow through orange to red. These are used as standards. If the addition of the same concentration of phenol red to a tube of bate liquor produces an orange-red color corresponding to that in the standard tube of pH = 7.9, then it is assumed that the pH value of the bate liquor is 7.9.

Clark and Lubs<sup>5</sup> have presented a series of indicators covering the range of pH values from 1.2 to 9.8. The common names and ranges covered are thymol blue 1.2 to 2.8, brom phenol blue 3.0 to 4.6, methyl red 4.4 to 6.0, brom cresol purple 5.2 to 6.8, brom thymol blue 6.0 to 7.6, phenol red 6.8 to 8.4, cresol red 7.2 to 8.8, thymol blue 8.0 to 9.6, cresol phthalein 8.2 to 9.8.

For details of the methods for preparing the color standards and of making the measurements under different conditions, the reader should consult Clark's book.<sup>5</sup> In the tannery, the indicator method must be looked upon as useful chiefly as a time saver in making routine measurements of a very few types of liquors, such as bate liquors and wash waters. Atkin and Thompson<sup>1</sup> have described its use with tan liquors. Wherever indicators are used in routine work, they should first be standardized against actual hydrogen electrode measurements.

For most measurements of pH value, the hydrogen electrode is necessary.

### **pH Values of Common Acid and Alkaline Solutions.**

In order to meet a demand for tables showing the pH values of acid and alkaline solutions at different concentrations, Thomas <sup>28</sup> computed and compiled from the literature a series of tables showing the degrees of ionization of a number of acids and bases commonly used in the tannery; a range from 0.001 to 2 molar is covered. These are incorporated into this chapter because they have been found very useful in tanning practice.

In making the calculations, Thomas used two modes of procedure. For the weak acids the concentrations of hydrogen ion have been calculated from the ionization constants (determined by conductivity measurements) by means of Ostwald's dilution law,

$$K = \frac{a^2}{V(1-a)}$$

where  $K$  is the ionization constant,  $V$  the volume in which 1 gram molecular weight is dissolved, and  $a$  the degree of ionization. By rearrangement of the equation, we get

$$a = \frac{-KV + \sqrt{K^2V^2 + 4KV}}{2}$$

But, since the value of  $K^2V^2$  is negligible compared to  $KV$ , it can be dropped for the purpose of making the calculations. The following expression, therefore, was used:

$$\text{Per cent ionization} = 100 \sqrt{KV} - 50KV.$$

For the strong acids, the experimentally determined values for  $100a$  at various concentrations were found in the literature. These were plotted against values for  $\log V$  and a smooth curve was drawn through the points. The desired values were then read from the curve. The hydroxide-ion concentrations of bases were obtained similarly.

The figures in the tables may be in error as much as 5 per cent, especially in the cases of the strong acids and bases, but they are the best obtainable at this time. They were obtained from conductivity data and not from measurements by the hydrogen electrode.



### Acids.

*Acetic Acid.*—Values calculated from the experimentally determined figures of Kendall.<sup>18</sup>

*Boric Acid.*—Calculated from  $K = 6.6 \times 10^{-10}$  at 25° C. by Lunden.<sup>19</sup> 0.8 molar is saturated solution and since this acid is exceedingly weak, only the concentrations at 0.8, 0.1, 0.01, and 0.001 molar are given in the table.

*Butyric Acid.*—For concentrations 2 to 0.1 molar, calculated from  $K = 1.49 \times 10^{-5}$  at 25° by Ostwald.<sup>24</sup> From 0.1 to 0.001 molar calculated from Ostwald's experimental values.

*Carbonic Acid.*—This acid is very weak and its concentration in solution depends upon the pressure of carbon dioxide on the surface of the solution. For this reason no special table was prepared and only two significant concentrations are given here, taken from Kendall.<sup>14</sup> At 25° the solubility of carbon dioxide in water at 1 atmosphere of pressure of carbon dioxide is 0.0337 mole per liter. The carbonic acid in this solution is 0.33 per cent ionized and hence its concentration of hydrogen ion is 0.00011 mole per liter, representing a pH value of 3.96. Under ordinary conditions, the partial pressure of carbon dioxide in the air is 0.000353 atmosphere, at which pressure carbon dioxide is soluble to the extent of 0.0000119 mole per liter, yielding a hydrogen-ion concentration of 0.000002 mole per liter or a pH value of 5.70.

*Citric Acid.*—For 2 to 0.4 molar, the values of Kendall, Booge and Andrews<sup>16</sup> are given. From 0.4 to 0.1 molar, the values are extrapolated. From 0.01 to 0.001 molar, the concentrations are calculated from the measurements of Walden.<sup>31</sup>

*Formic Acid.*—From 2 to 0.1 molar, the values are calculated from  $K = 21.4 \times 10^{-5}$  at 25°, as given by Ostwald.<sup>24</sup> From 0.1 to 0.001 molar, they are calculated from Ostwald's experimental determinations.

*Gallic Acid.*—From 1 to 0.03 molar, values are calculated from  $K = 4.0 \times 10^{-5}$ , as given by Ostwald.<sup>24</sup> From 0.03 to 0.001 molar, values are calculated from Ostwald's experimental values.

*Hydrochloric Acid.*—The figures for 2 to 0.5 molar are from Jones.<sup>12</sup> Those for 0.5 to 0.001 molar are calculated from Kohlrausch's<sup>21</sup> experimentally determined values.

*Lactic Acid.*—The figures for 2 to 0.1 molar are based upon the figures of Kendall, Booge and Andrews;<sup>15</sup> those for 0.1 to 0.001 molar are calculated from the experimental values of Ostwald.<sup>24</sup>

*Nitric Acid.*—The 2 to 1 molar values are taken from Jones;<sup>12</sup> those for 0.5 to 0.001 molar are calculated from Kohlrausch's<sup>21</sup> data.

*Oxalic Acid.*—The only data available are those of Ostwald,<sup>24</sup> covering the range only from 0.03 to 0.004 molar. This acid is too highly ionized to permit calculations by the dilution law.

*Phosphoric Acid.*—Figures for 2 to 0.1 molar are calculated from the data of Kendall, Booge and Andrews;<sup>15</sup> those from 0.1 to 0.001 molar from the experimental data of Noyes and Eastman.<sup>22</sup>

*Salicylic Acid.*—Values are based upon the experimental data of Kendall.<sup>13</sup> 0.0167 molar represents the limit of solubility.

*Sulfuric Acid.*—The figures for 2 to 1 molar are from Jones;<sup>12</sup> those for 0.5 to 0.001 molar from the experimental data of Kohlrausch.<sup>21</sup>

*Tartaric Acid.*—From 2 to 0.04 molar, the figures are calculated from the data of Kendall, Booge and Andrews;<sup>15</sup> from 0.04 to 0.001 molar, they are calculated from Ostwald's<sup>24</sup> experimental data.

### Bases.

*Ammonium Hydroxide.*—The figures for this weak base are calculated, by means of the dilution law, from  $K = 1.8 \times 10^{-5}$  at 25° C., as given by Noyes, Kato and Sosman.<sup>23</sup>

*Barium Hydroxide.*—The only available data for this base are those of Noyes and Eastman,<sup>22</sup> which range from 0.001 to 0.05 molar, upon which the calculations in the table are based.

*Calcium Hydroxide.*—No series of experimental data for this base could be found, but it is so similar to barium hydroxide that probably no great error would arise from the use of the barium hydroxide figures.

*Potassium Hydroxide.*—The 2 molar value is from Jones.<sup>12</sup> Values for 1 to 0.4 molar and from 0.03 to 0.001 molar are calculated from Kohlrausch's<sup>21</sup> data; those between 0.4 and 0.03 molar are obtained by extrapolation.

*Sodium Hydroxide.*—The 2 molar figure is from Jones;<sup>12</sup> the others are from Kohlrausch's<sup>21</sup> data.

### Order of Strengths.

Listing the acids in order of increasing strength, or hydrogen-ion activities, we have

Boric  
Carbonic  
Butyric  
Acetic  
Gallic  
Lactic  
Formic  
Citric  
Tartaric  
Salicylic  
Phosphoric  
Oxalic  
Sulfuric  
Nitric, Hydrochloric

Boric is the weakest acid in the list and hydrochloric and nitric are the strongest.

The bases in order of decreasing hydroxide-ion activity are

Potassium hydroxide  
Sodium hydroxide  
Barium hydroxide, Calcium hydroxide  
Ammonium hydroxide

### Temperature.

All of the values given in Tables V to XIII are for a temperature of 25° C. The temperature coefficient of ionization is small enough to be neglected for most practical purposes. The figures may, therefore, be considered valid for the range of temperature found in the tannery.

The pH values corresponding to the various hydrogen-ion concentrations have been added to Thomas' tables in order to increase their usefulness.

TABLE V.

Moles of Acid Per Liter	Hydrochloric Acid			Nitric Acid		
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value
0.001.....	100.0	0.0010	3.00	100.0	0.0010	3.00
0.002.....	100.0	0.0020	2.70	99.5	0.0020	2.70
0.003.....	100.0	0.0030	2.52	99.5	0.0030	2.52
0.004.....	100.0	0.0040	2.40	99.4	0.0040	2.40
0.005.....	100.0	0.0050	2.30	99.4	0.0050	2.30
0.006.....	100.0	0.0060	2.22	99.4	0.0060	2.22
0.007.....	100.0	0.0070	2.15	99.3	0.0070	2.15
0.008.....	100.0	0.0080	2.10	99.3	0.0079	2.10
0.009.....	99.9	0.0090	2.05	99.3	0.0089	2.05
0.01.....	99.8	0.010	2.00	99.3	0.010	2.00
0.02.....	98.8	0.020	1.70	99.3	0.020	1.70
0.03.....	98.0	0.029	1.54	99.2	0.030	1.52
0.04.....	97.6	0.039	1.41	98.7	0.039	1.41
0.05.....	96.8	0.048	1.32	98.3	0.049	1.31
0.06.....	96.4	0.058	1.24	97.6	0.059	1.23
0.07.....	95.8	0.067	1.17	97.3	0.068	1.17
0.08.....	95.6	0.076	1.12	96.8	0.077	1.11
0.09.....	95.2	0.086	1.07	96.3	0.087	1.06
0.1.....	94.8	0.095	1.02	96.0	0.096	1.02
0.2.....	92.0	0.184	0.74	92.9	0.186	0.73
0.3.....	90.1	0.270	0.57	90.7	0.272	0.57
0.4.....	88.7	0.355	0.45	89.4	0.358	0.45
0.5.....	87.5	0.438	0.36	87.9	0.439	0.36
0.6.....	86.5	0.519	0.28	...	....	...
0.7.....	84.7	0.593	0.23	...	....	...
0.8.....	83.3	0.666	0.18	...	....	...
0.9.....	81.5	0.734	0.13	...	....	...
1.0.....	79.6	0.796	0.10	84.8	0.848	0.07
2.0.....	69.3	1.386	-0.14	73.9	1.478	-0.17

TABLE VI.

Moles of Acid Per Liter	Sulfuric Acid *			Phosphoric Acid †		
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value
0.001.....	97.7	0.0020	2.70	89.0	0.0009	3.05
0.002.....	94.7	0.0038	2.42	83.0	0.0017	2.77
0.003.....	90.5	0.0054	2.27	77.5	0.0023	2.64
0.004.....	88.0	0.0070	2.15	73.5	0.0029	2.54
0.005.....	85.9	0.0086	2.07	70.0	0.0035	2.46
0.006.....	84.2	0.0101	2.00	67.5	0.0041	2.39
0.007.....	82.7	0.0116	1.94	65.0	0.0046	2.34
0.008.....	81.8	0.0131	1.88	63.0	0.0050	2.30
0.009.....	80.5	0.0145	1.84	60.5	0.0054	2.27
0.01.....	79.6	0.016	1.80	59.0	0.006	2.23
0.02.....	73.1	0.029	1.54	47.5	0.010	2.00
0.03.....	69.4	0.042	1.38	42.0	0.013	1.89
0.04.....	66.8	0.053	1.28	38.0	0.015	1.82
0.05.....	64.8	0.065	1.19	35.0	0.018	1.74
0.06.....	63.5	0.076	1.12	33.0	0.020	1.70
0.07.....	62.4	0.087	1.06	31.0	0.022	1.66
0.08.....	61.7	0.099	1.00	30.0	0.024	1.62
0.09.....	61.1	0.110	0.96	28.5	0.026	1.58
0.1.....	60.7	0.121	0.92	27.5	0.028	1.55
0.2.....	57.6	0.230	0.64	22.8	0.046	1.34
0.3.....	56.0	0.336	0.47	20.7	0.062	1.21
0.4.....	54.7	0.438	0.36	19.8	0.079	1.10
0.5.....	53.6	0.536	0.27	19.0	0.095	1.02
0.6.....	52.9	0.635	0.20	18.8	0.113	0.95
0.7.....	52.0	0.728	0.14	18.0	0.126	0.90
0.8.....	51.4	0.822	0.09	17.9	0.143	0.84
0.9.....	50.9	0.916	0.04	17.7	0.159	0.80
1.0.....	50.7	1.014	-0.01	17.5	0.175	0.76
2.0.....	39.9	1.596	-0.20	16.1	0.322	0.49

\* 100 per cent ionization taken as complete ionization into H<sup>+</sup>, H<sup>+</sup>, and SO<sub>4</sub><sup>2-</sup>.† 100 per cent ionization taken as complete ionization into H<sup>+</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup>.

TABLE VII.

Moles of Acid Per Liter	Formic Acid				Acetic Acid			
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value		
0.001.....	35.8	0.00036	3.44	12.8	0.00013	3.89		
0.002.....	27.1	0.00054	3.27	9.2	0.00018	3.74		
0.003.....	22.0	0.00066	3.18	7.5	0.00023	3.64		
0.004.....	20.1	0.00080	3.10	6.6	0.00026	3.58		
0.005.....	18.0	0.00090	3.05	5.9	0.00030	3.52		
0.006.....	16.6	0.00100	3.00	5.4	0.00032	3.49		
0.007.....	15.5	0.00109	2.96	5.0	0.00035	3.46		
0.008.....	14.8	0.00118	2.93	4.7	0.00038	3.42		
0.009.....	14.0	0.00126	2.90	4.4	0.00040	3.40		
0.01.....	13.4	0.0013	2.87	4.2	0.00042	3.38		
0.02.....	9.7	0.0019	2.72	3.0	0.00060	3.22		
0.03.....	8.1	0.0024	2.62	2.4	0.00072	3.14		
0.04.....	7.1	0.0028	2.55	2.1	0.00084	3.08		
0.05.....	6.4	0.0032	2.49	1.9	0.00095	3.02		
0.06.....	5.8	0.0035	2.46	1.7	0.00102	2.99		
0.07.....	5.4	0.0038	2.42	1.55	0.00109	2.96		
0.08.....	5.0	0.0040	2.40	1.5	0.00120	2.92		
0.09.....	4.7	0.0042	2.38	1.4	0.00126	2.90		
0.1.....	4.5	0.0045	2.35	1.3	0.00130	2.89		
0.2.....	3.2	0.0064	2.19	0.9	0.00180	2.74		
0.3.....	2.6	0.0078	2.11	0.7	0.00210	2.68		
0.4.....	2.3	0.0092	2.04	0.6	0.00240	2.62		
0.5.....	2.1	0.0105	1.98	0.57	0.00285	2.55		
0.6.....	1.9	0.0114	1.94	0.50	0.00300	2.52		
0.7.....	1.8	0.0126	1.90	0.45	0.00315	2.50		
0.8.....	1.7	0.0136	1.87	0.42	0.00336	2.47		
0.9.....	1.6	0.0144	1.84	0.40	0.00360	2.44		
1.0.....	1.5	0.0150	1.82	0.37	0.00370	2.43		
2.0.....	1.03	0.0206	1.69	0.30	0.00600	2.22		

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TABLE VIII.

Moles of Acid Per Liter	Gallic Acid				Lactic Acid	
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value
0.001.....	18.7	0.00019	3.72	30.9	0.00031	3.51
0.002.....	13.4	0.00027	3.57	23.0	0.00046	3.34
0.003.....	10.7	0.00032	3.49	18.7	0.00056	3.25
0.004.....	9.3	0.00037	3.43	16.7	0.00067	3.18
0.005.....	8.4	0.00042	3.38	15.1	0.00076	3.12
0.006.....	7.6	0.00046	3.34	13.9	0.00083	3.08
0.007.....	7.0	0.00049	3.31	12.9	0.00090	3.05
0.008.....	6.7	0.00054	3.27	12.2	0.00098	3.01
0.009.....	6.2	0.00056	3.25	11.5	0.00104	2.98
0.01.....	5.9	0.00059	3.23	11.0	0.00110	2.96
0.02.....	4.1	0.00082	3.09	8.0	0.00160	2.80
0.03.....	3.3	0.00099	3.00	6.6	0.00198	2.70
0.04.....	3.0	0.00120	2.92	5.8	0.00232	2.63
0.05.....	2.70	0.00135	2.87	5.2	0.00260	2.58
0.06.....	2.50	0.00150	2.82	4.8	0.00288	2.54
0.07.....	2.30	0.00161	2.79	4.3	0.00301	2.52
0.08.....	2.20	0.00176	2.75	4.1	0.00328	2.48
0.09.....	2.05	0.00185	2.73	3.8	0.00342	2.47
0.1.....	1.98	0.0020	2.70	3.7	0.00370	2.43
0.2.....	1.40	0.0028	2.55	2.7	0.0054	2.27
0.3.....	1.15	0.0035	2.46	2.2	0.0066	2.18
0.4.....	1.00	0.0040	2.40	1.8	0.0072	2.14
0.5.....	0.89	0.0045	2.35	1.6	0.0080	2.10
0.6.....	0.80	0.0048	2.32	1.5	0.0090	2.05
0.7.....	0.74	0.0052	2.28	1.4	0.0098	2.01
0.8.....	0.70	0.0056	2.25	1.3	0.0104	1.98
0.9.....	0.68	0.0061	2.21	1.2	0.0108	1.97
1.0.....	0.63	0.0063	2.20	1.1	0.0110	1.96
2.0.....	...	.....	...	0.8	0.0160	1.80

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TABLE IX.

Moles of Acid Per Liter	Butyric Acid			Boric Acid *		
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value
0.001.....	11.4	0.00011	3.96	0.080	0.0000008	6.10
0.002.....	8.3	0.00017	3.77	....	.....	...
0.003.....	6.8	0.00020	3.70	....	.....	...
0.004.....	6.0	0.00024	3.62	....	.....	...
0.005.....	5.4	0.00027	3.57	....	.....	...
0.006.....	4.9	0.00029	3.54	....	.....	...
0.007.....	4.55	0.00032	3.49	....	.....	...
0.008.....	4.3	0.00034	3.47	....	.....	...
0.009.....	3.95	0.00036	3.44	....	.....	...
0.01.....	3.8	0.00038	3.42	0.026	0.0000026	5.58
0.02.....	2.7	0.00054	3.27	....	.....	...
0.03.....	2.2	0.00066	3.18	....	.....	...
0.04.....	1.95	0.00078	3.11	....	.....	...
0.05.....	1.7	0.00085	3.07	....	.....	...
0.06.....	1.6	0.00096	3.02	....	.....	...
0.07.....	1.4	0.00098	3.01	....	.....	...
0.08.....	1.35	0.00108	2.97	....	.....	...
0.09.....	1.25	0.00113	2.95	....	.....	...
0.1.....	1.2	0.00120	2.92	0.008	0.0000080	5.10
0.2.....	0.86	0.00172	2.76	....	.....	...
0.3.....	0.70	0.00210	2.68	....	.....	...
0.4.....	0.60	0.00240	2.62	....	.....	...
0.5.....	0.54	0.00270	2.57	....	.....	...
0.6.....	0.49	0.00294	2.53	....	.....	...
0.7.....	0.43	0.00301	2.52	....	.....	...
0.8.....	0.41	0.00328	2.48	0.003	0.0000240	4.62
0.9.....	0.40	0.00360	2.44	....	.....	...
1.0.....	0.39	0.00390	2.40	....	.....	...
2.0.....	0.27	0.00540	2.27	....	.....	...

\* 100 per cent ionization taken as complete ionization into H<sup>+</sup> and H<sub>2</sub>BO<sub>3</sub>'.



TABLE X.

Moles of Acid Per Liter	Tartaric Acid *			Citric Acid †		
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value
0.001.....	65.3	0.0007	3.15	60.2	0.0006	3.22
0.002.....	51.0	0.0010	3.00	47.4	0.0009	3.05
0.003.....	43.0	0.0013	2.89	39.8	0.0012	2.92
0.004.....	39.0	0.0016	2.80	36.0	0.0014	2.85
0.005.....	35.5	0.0018	2.74	33.1	0.0017	2.77
0.006.....	33.0	0.0020	2.70	30.8	0.0018	2.74
0.007.....	31.0	0.0022	2.66	28.9	0.0020	2.70
0.008.....	30.0	0.0024	2.62	27.6	0.0022	2.66
0.009.....	28.0	0.0025	2.60	25.9	0.0023	2.64
0.01.....	27.0	0.0027	2.57	25.0	0.0025	2.60
0.02.....	19.5	0.0039	2.41	18.3	0.0037	2.43
0.03.....	16.5	0.0050	2.30	15.5	0.0047	2.33
0.04.....	14.5	0.0058	2.24	13.8	0.0055	2.26
0.05.....	13.1	0.0066	2.18	12.5	0.0063	2.20
0.06.....	12.2	0.0073	2.14	11.5	0.0069	2.16
0.07.....	11.4	0.0080	2.10	10.7	0.0075	2.12
0.08.....	10.9	0.0087	2.06	10.1	0.0081	2.09
0.09.....	10.2	0.0092	2.04	9.5	0.0086	2.07
0.1.....	9.9	0.010	2.00	9.1	0.009	2.04
0.2.....	7.1	0.014	1.85	6.1	0.012	1.92
0.3.....	5.7	0.017	1.77	4.7	0.014	1.85
0.4.....	4.9	0.020	1.70	4.0	0.016	1.80
0.5.....	4.2	0.021	1.68	3.5	0.018	1.74
0.6.....	3.7	0.022	1.66	3.1	0.019	1.72
0.7.....	3.5	0.025	1.60	3.0	0.021	1.68
0.8.....	3.2	0.026	1.58	2.9	0.023	1.64
0.9.....	3.0	0.027	1.57	2.8	0.025	1.60
1.0.....	2.9	0.029	1.54	2.7	0.027	1.57
2.0.....	2.1	0.042	1.38	1.8	0.036	1.44

\* 100 per cent ionization taken as complete ionization into H<sup>+</sup> and HC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>.† 100 per cent ionization taken as complete ionization into H<sup>+</sup> and H<sub>2</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>.

TABLE XI.

Moles of Acid Per Liter	Oxalic Acid *			Salicylic Acid		
	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value	Per Cent Ionized	Moles H <sup>+</sup> Per Liter	pH Value
0.001.....	...	.....	...	62.0	0.0006	3.22
0.002.....	...	.....	...	51.0	0.0010	3.00
0.003.....	...	.....	...	44.5	0.0013	2.89
0.004.....	95.0	0.0038	2.42	40.0	0.0016	2.80
0.005.....	93.0	0.0047	2.33	37.0	0.0019	2.72
0.006.....	91.5	0.0055	2.26	34.5	0.0021	2.68
0.007.....	90.0	0.0063	2.20	32.0	0.0022	2.66
0.008.....	89.0	0.0071	2.15	30.5	0.0024	2.62
0.009.....	88.0	0.0079	2.10	29.0	0.0026	2.58
0.010.....	87.0	0.0087	2.06	27.7	0.0028	2.55
0.0167.....	...	.....	...	24.0	0.0040	2.40
0.020.....	79.0	0.0158	1.80	...	.....	...
0.030.....	73.5	0.0221	1.66	...	.....	...

\* 100 per cent ionization taken as complete ionization into H<sup>+</sup> and HC<sub>2</sub>O<sub>4</sub>.

TABLE XII.

Moles of Base Per Liter	Potassium Hydroxide			Sodium Hydroxide		
	Per Cent Ionized	Moles OH' Per Liter	pH Value	Per Cent Ionized	Moles OH' Per Liter	pH Value
0.001.....	100.0	0.001	11.00	100.0	0.001	11.00
0.002.....	100.0	0.002	11.30	100.0	0.002	11.30
0.003.....	100.0	0.003	11.48	100.0	0.003	11.48
0.004.....	100.0	0.004	11.60	100.0	0.004	11.60
0.005.....	100.0	0.005	11.70	100.0	0.005	11.70
0.006.....	100.0	0.006	11.78	100.0	0.006	11.78
0.007.....	100.0	0.007	11.85	100.0	0.007	11.85
0.008.....	100.0	0.008	11.90	99.9	0.008	11.90
0.009.....	99.9	0.009	11.95	99.7	0.009	11.95
0.01.....	99.9	0.010	12.00	99.5	0.010	12.00
0.02.....	99.3	0.020	12.30	97.9	0.020	12.30
0.03.....	98.7	0.030	12.48	96.8	0.029	12.46
0.04.....	97.9	0.039	12.59	96.0	0.038	12.58
0.05.....	97.3	0.049	12.69	95.3	0.048	12.68
0.06.....	96.7	0.058	12.76	94.7	0.057	12.76
0.07.....	96.2	0.067	12.83	94.1	0.066	12.82
0.08.....	95.8	0.077	12.89	93.7	0.075	12.88
0.09.....	95.3	0.086	12.93	93.2	0.084	12.92
0.1.....	95.0	0.095	12.98	92.9	0.093	12.97
0.2.....	92.2	0.184	13.26	89.8	0.180	13.26
0.3.....	90.1	0.270	13.43	87.0	0.261	13.42
0.4.....	88.8	0.355	13.55	85.3	0.341	13.53
0.5.....	87.6	0.438	13.64	83.5	0.418	13.62
0.6.....	86.3	0.518	13.71	81.9	0.491	13.69
0.7.....	85.0	0.595	13.77	80.4	0.563	13.75
0.8.....	84.3	0.674	13.83	79.2	0.634	13.80
0.9.....	82.8	0.745	13.87	77.7	0.699	13.84
1.0.....	81.9	0.819	13.91	76.6	0.766	13.88
2.0.....	66.3	1.326	14.12	57.0	1.140	14.06

TABLE XIII.

Moles of Base Per Liter	Ammonium Hydroxide			Barium Hydroxide *		
	Per Cent Ionized	Moles OH' Per Liter	pH Value	Per Cent Ionized	Moles OH' Per Liter	pH Value
0.001.....	12.52	0.00013	10.11	96.0	0.0010	11.00
0.002..	8.99	0.00018	10.26	95.0	0.0019	11.28
0.003..	7.44	0.00022	10.34	94.0	0.0028	11.45
0.004.....	6.48	0.00026	10.42	93.0	0.0037	11.57
0.005.....	5.82	0.00029	10.46	92.0	0.0046	11.66
0.006.....	5.33	0.00032	10.51	91.3	0.0055	11.74
0.007.....	4.93	0.00035	10.54	91.0	0.006	11.78
0.008.....	4.62	0.00037	10.57	90.5	0.007	11.85
0.009.....	4.37	0.00039	10.59	90.0	0.008	11.90
0.01.....	4.15	0.00042	10.62	88.4	0.009	11.95
0.02.....	2.96	0.00059	10.77	86.0	0.017	12.23
0.03.....	2.42	0.00073	10.86	82.8	0.025	12.40
0.04.....	2.12	0.00085	10.93	81.0	0.032	12.51
0.05.....	1.88	0.00094	10.97	80.0	0.040	12.60
0.06.....	1.72	0.00103	11.01	...	....	....
0.07.....	1.59	0.00111	11.05	...	....	....
0.08.....	1.49	0.00119	11.08	...	....	....
0.09.....	1.40	0.00126	11.10	...	....	....
0.1.....	1.33	0.00133	11.12	...	....	....
0.2.....	0.94	0.00188	11.27	...	....	....
0.3.....	0.77	0.00231	11.36	...	....	....
0.4.....	0.67	0.00268	11.43	...	....	....
0.5.....	0.60	0.00300	11.48	...	....	....
0.6.....	0.55	0.00330	11.52	...	....	....
0.7.....	0.50	0.00350	11.54	...	....	....
0.8.....	0.47	0.00376	11.58	...	....	....
0.9.....	0.45	0.00405	11.61	...	....	....
1.0.....	0.42	0.00420	11.62	...	....	....
2.0.....	0.30	0.00600	11.78	...	....	....

\* 100 per cent ionization taken as complete ionization into  $\text{BaOH}^+$  and  $\text{OH}'$ .

NOTE: Where figures for calcium hydroxide are desired, it is suggested that those for barium hydroxide be used.

### Effect of Added Salts.

The figures given in the tables are for pure solutions of the acids or bases. Measurements with the hydrogen electrode indicate that the addition of sodium chloride, or other neutral chlorides, tends to increase the hydrogen-ion concentrations of acids<sup>7, 10, 26, 29</sup> and the hydroxide-ion concentration of bases.<sup>10</sup> Neutral sulfates, on the other hand, tend to decrease the hydrogen-ion concentrations of acids.

This contrasting effect of chlorides and sulfates on the hydrogen-ion concentrations of solutions of sulfuric and hydrochloric acids was shown by Thomas and Baldwin.<sup>30</sup> Their results for 0.1 normal acids

are shown in Figs. 5 and 6. In each case a solution of acid was mixed with a solution of salt and diluted to 100 cubic centimeters so that the final concentration of acid was 0.1 normal, while the concentration of salt was varied. The hydrogen-ion concentrations were measured, by means of the hydrogen electrode, two days after the solutions were made up.

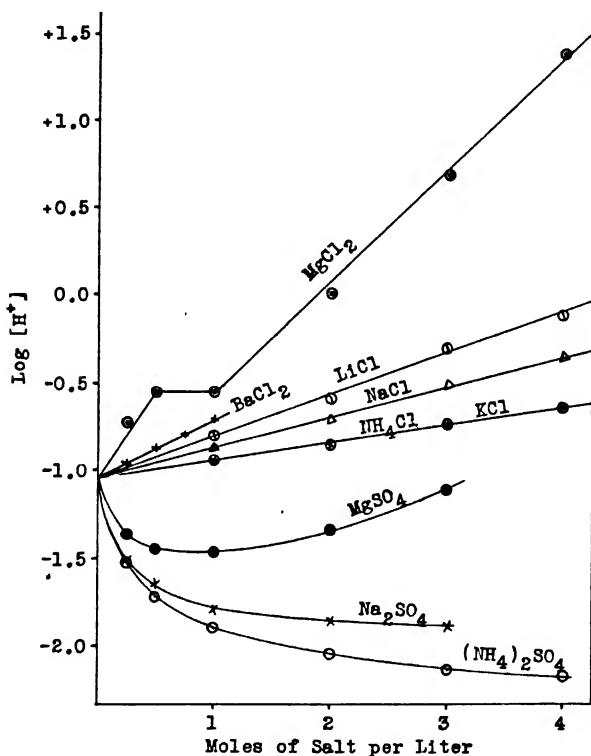
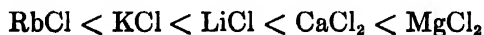


FIG. 5.—Effect of concentration of various salts upon the hydrogen-ion concentration of tenth-normal hydrochloric acid solution.

When the chlorides are arranged in order of their ability to increase the hydrogen-ion concentration, the following series is obtained:



But this is also the order of increasing degree of hydration, or the number of molecules of water combined with the individual cations at infinite dilution. Poma<sup>26</sup> found that chlorides increase the hydrogen-ion concentrations of hydrochloric acid solutions in the following order:



In extending the work of Thomas and Baldwin, Wilson<sup>22</sup> pointed out that one of the remarkable features of their results is that when the logarithm of the concentration of hydrogen ion is plotted against the concentration of added salt, in the case of the alkali chlorides, the curves are apparently straight lines, of the general formula

$$\log [H^+] = \log a + bm$$

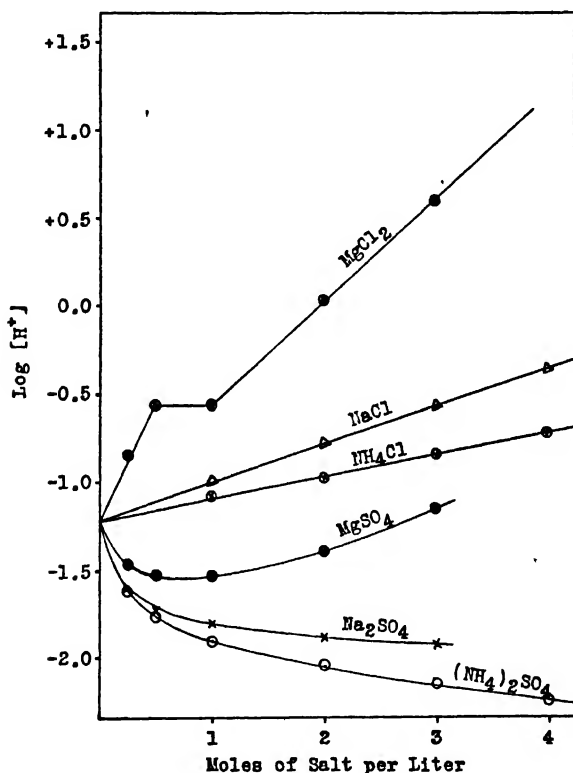


FIG. 6.—Effect of concentration of various salts upon the hydrogen-ion concentration of tenth-normal sulfuric acid solution.

where  $b$  is a constant,  $a$  the hydrogen-ion concentration when no salt is present, and  $[H^+]$  the hydrogen-ion concentration in the presence of  $m$  moles per liter of salt.

It was also shown that this equation is independent of the strength of the acid solution, the value for  $b$  depending only upon the kind of alkali chloride added. Curves showing the effect of adding sodium chloride to four different concentrations of sulfuric acid are shown

in Fig. 7. Apparently the curves are not only straight lines, but all four have the same slope, the average value for  $b$  being 0.205.

The addition of 4 moles per liter of sodium chloride raises the hydrogen-ion concentration of 0.1 molar hydrochloric acid to 0.44 mole per liter, which can be accounted for only on the assumption that more than three-quarters of the water present has ceased to play the rôle of solvent. The hydration theory assumes that this is brought about by the water combining with the salt.

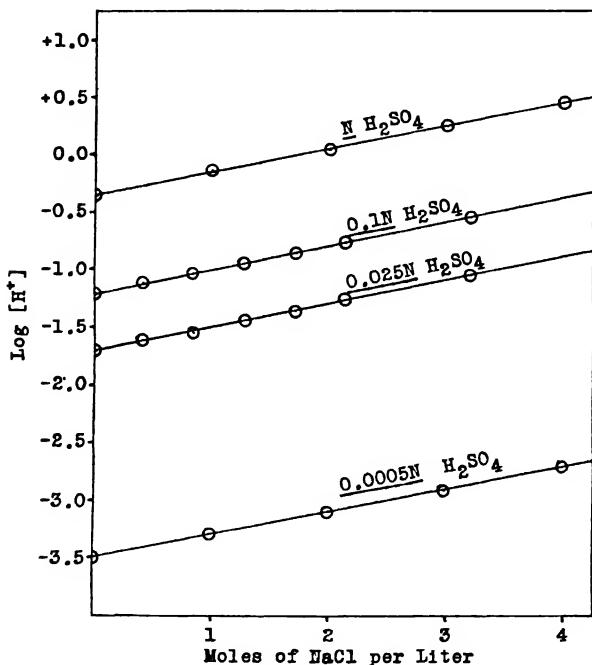


Fig. 7.—Effect of concentration of sodium chloride upon the hydrogen-ion concentration of various strengths of sulfuric acid solution.

If the rise in hydrogen-ion concentration is due to the removal of water by the added sodium chloride, it should be possible to determine the degree of hydration of the salt at any concentration from hydrogen-ion measurements. Assuming this to be so, we should reason as follows: From the above equation,  $\log([H^+]/a) = bm$ . But  $[H^+]/a$  is the factor by which the acid concentration has been multiplied by adding  $m$  moles per liter of salt. Let  $w$  represent the total number of moles of water, free or combined with salt, in 1 liter of solution containing  $m$  moles of salt. The moles of free water then

equal  $w_a/[H^+]$  and the moles of water combined with one mole of salt equal  $(w/m) \times (1 - a/[H^+])$ . Calling this latter value  $h$ , we have

$$h = w(1 - 10^{-bm})/m.$$

From this, hydration values can be calculated for any concentration of salt. For infinite dilution of salt, the expression becomes greatly simplified, for

$$\lim_{m=0} (1 - 10^{-bm})/m = 2.30b.$$

But at infinite dilution  $w = 55.5$  and hence

$$h = 128b.$$

The calculated number of molecules of water combined with one molecule of sodium chloride at infinite dilution would thus be  $128 \times 0.205$  or 26.2, which is in striking agreement with the value 26.5 obtained by Smith<sup>27</sup> from a very different type of measurement. Calculations of the degrees of hydration at infinite dilution of the chlorides of potassium, ammonium, and lithium made from the equation  $h = 128b$  also agreed fairly well with Smith's corresponding values.

A means is thus afforded to calculate the change of pH value that will be produced by the addition of a neutral chloride to an acid solution. Let  $I$  represent the pH value of the acid solution containing no salt, which may be found in the preceding tables. Let  $F$  be the pH value after the addition of  $m$  moles per liter of salt and  $H$  be the number of molecules of water combined with one molecule of salt at infinite dilution. Then

$$F = I - 0.0078Hm.$$

The use of this equation does not depend upon the validity of the theory. The measurements of Thomas and Baldwin show that it may be used for the addition of chlorides to sulfuric and hydrochloric acids by substituting the following values for  $H$ :

potassium chloride	15
ammonium chloride	15
sodium chloride	26
lithium chloride	35
barium chloride	50

The effect of adding sulfates cannot, however, be attributed to hydration, since they decrease the hydrogen-ion concentrations of acid





## Chapter 5.

### Physical Chemistry of the Proteins.

One of the foundations upon which leather chemistry is built is the physical chemistry of the proteins. Until comparatively recently our knowledge of the chemical reactions of the proteins was hardly sufficient to permit any satisfactory quantitative treatment. Many of the important reactions of proteins seemed to run contrary to the well-established principles of physical chemistry until chemists discovered that their observations had been incomplete; the seeming deviations were merely the result of the operation of unrecognized variable factors. One of these was the hydrogen-ion concentration. Another cause of seeming deviations was the failure to recognize the full number of phases existing in a given system.

A great advance in the quantitative development of the physical chemistry of the proteins was made by Donnan's <sup>9, 10, 25</sup> theory of membrane equilibria, which was applied by Procter <sup>55</sup> to the swelling of gelatin and further developed by Procter and Wilson <sup>56</sup> into a quantitative theory of the swelling of protein jellies. In an extensive series of researches, Loeb <sup>38</sup> has extended this work to include also the osmotic pressure, viscosity, stability, and electrical potential differences of protein systems as well as a general theory of colloidal behavior. This valuable work should be consulted as having an important bearing upon leather chemistry.

Because of the very great importance of the Donnan theory, it will be discussed in this chapter in detail. It does not explain all of the important reactions of the proteins, but it is necessary to an understanding of certain phenomena shown by the proteins. It has made possible quantitative explanations of the swelling of protein jellies, of variations in viscosity of gelatin dispersions, of the osmotic pressures developed by certain protein systems, of the electrical potential differences observed between the different phases in a protein system, and of the stability of certain dispersions. In the enthusiasm naturally aroused by the development of a theory so important, false impressions were spread as to the scope of the Donnan theory and chemists

began to point out protein reactions that could not be explained by it. They showed that in many reactions different ions of the same valency had very different effects upon proteins, whereas there are no specific effects of ions of the same valency involved in the phenomena explained by the Donnan theory. The theory does not attempt to explain why the bromide ion is so much more active in dispersing collagen than the chloride ion or any other reaction not clearly defined by the theory.

### Donnan's Theory of Membrane Equilibria.

This theory deals with the equilibria resulting from the separation by a membrane of two solutions, one of which contains an ionogen having one ion that cannot diffuse through the membrane, which is permeable to all other ions of the system. As an example, Donnan takes an aqueous solutions of a salt NaR, such as Congo red, in contact with a membrane which is impermeable to the anion R' and the non-ionized salt, but will allow Na<sup>+</sup> or any other ion to pass freely through it.

$[Na^+] = a$ $[R'] = a$ Solution I	$[Na^+] = a$ $[Cl'] = a$ Solution II
Initial State	
$[Na^+] = a$ $[R'] = a$ $[Na^+] = a - b$ $[Cl'] = a - b$ Solution I	$[Na^+] = b$ $[Cl'] = b$ Solution II
Equilibrium State	

Fig. 8.—Diagrams of a simple example of the operation of the Donnan equilibrium.

Fig. 8 represents the initial and equilibrium states of a very simple example. In the initial state the membrane separates two solutions of equal and constant volume, Solution I containing  $a$  moles per liter of completely ionized NaR and Solution II  $a$  moles per liter of completely ionized NaCl. The membrane is permeable to Na<sup>+</sup> and to Cl', but not to R'. The impermeability of the membrane to R' initially prevents any diffusion from Solution I to Solution II, since any diffusion of sodium ions would immediately set up powerful electrostatic forces preventing further diffusion. But there is no such restraint on the diffusion of sodium and chloride ions from II to I.

When equilibrium is finally established, let  $b$  represent the concentration to which the ionized sodium chloride in Solution II has been reduced by its diffusion into Solution I. If there were no NaR present in Solution I, sodium chloride would diffuse into it until the concentration was the same on both sides of the membrane, or  $a - b = b = a/2$ . It is the presence of the NaR that makes this uniform distribution of sodium chloride throughout the whole system impossible and brings about what has come to be known as a Donnan equilibrium. The total final concentration of sodium ion in Solution I is  $a + a - b$ , or  $2a - b$ . But the concentration of chloride ion is only  $a - b$ . On the other hand, sodium and chloride ions are present in Solution II in equal concentration.

Donnan showed how to calculate the relation existing between the concentration of sodium chloride in Solution I and that in Solution II at equilibrium by a system of relatively simple thermodynamic reasoning. It should be noted that the reasoning is independent of the relative volumes of the two solutions and of the initial concentrations of NaR in the one solution and NaCl in the other. When equilibrium is established, if a small virtual change is made reversibly at constant temperature and volume, the free energy will remain unchanged; that is, no work will be done. The change here considered is the transfer of  $dn$  moles of  $\text{Na}^+$  and  $\text{Cl}'$  from II to I. The work, which equals zero, is

$$dn \cdot RT \cdot \log_e \frac{[\text{Na}^+]_{\text{II}}}{[\text{Na}^+]_{\text{I}}} + dn \cdot RT \cdot \log_e \frac{[\text{Cl}']_{\text{II}}}{[\text{Cl}']_{\text{I}}} = 0,$$

whence  $[\text{Na}^+]_{\text{II}} \times [\text{Cl}']_{\text{II}} = [\text{Na}^+]_{\text{I}} \times [\text{Cl}']_{\text{I}}$ .

(The brackets indicate concentration in moles per liter.)

Equilibrium will be established only when the product of the concentrations of  $\text{Na}^+$  and  $\text{Cl}'$  has the same value on both sides of the membrane.

In the simple case pictured in Fig. 8,

$$(2a - b) \times (a - b) = b^2,$$

or

$$2a = 3b.$$

At equilibrium one-third of the sodium chloride will be in Solution I and two-thirds will be left in Solution II, further diffusion into Solution I having been prevented by the presence of the NaR. The final concentrations of the ions will be as follows: in Solution I, total  $[\text{Na}^+] = 4a/3$ ,  $[\text{R}'] = a$ , and  $[\text{Cl}'] = a/3$ ; in Solution II,  $[\text{Na}^+] = [\text{Cl}'] = 2a/3$ .

The equation of products, derived thermodynamically, is a general one and is true for all equilibria of this type. It is of such fundamental importance in the quantitative development of leather chemistry that any doubt as to its validity should be dispelled at the outset. The derivation of the equation need not involve the use of thermodynamics, since it can readily be visualized. In passing from one phase to the other, the oppositely charged ions must move in pairs, since they would otherwise set up powerful electrostatic forces that would prevent their free diffusion. For this reason a sodium or a chlorine ion striking the membrane alone could not pass through it. But, since the membrane is freely permeable to both  $\text{Na}^+$  and  $\text{Cl}'$ , when two oppositely charged ions strike the membrane together, there is nothing to prevent them from passing through into the solution on the opposite side. The rate of transfer of these ions from one solution to the other depends, therefore, upon the frequency with which they chance to strike the membrane in pairs, which is measured by the product of their concentrations. At equilibrium the rate of transfer of  $\text{Na}^+$  and  $\text{Cl}'$  from Solution II to Solution I exactly equals the rate of transfer of these ions from Solution I to Solution II, from which it follows that the product of the concentrations of these ions has the same value in both solutions.

It is interesting now to note the effect of complicating the system by the introduction of another salt, such as  $\text{KBr}$ . Following the same line of reasoning, it will be evident that equilibrium will be established only when the product  $[\text{K}^+] \times [\text{Br}']$  has the same value in both solutions, and the same is true for the products  $[\text{K}^+] \times [\text{Cl}']$  and  $[\text{Na}^+] \times [\text{Br}']$ . In fact, with any number of mono-monovalent ionogens present in the system, the product of the concentrations of any pair of diffusible and oppositely charged ions will have the same value in both solutions.

Introducing polyvalent ions into the system makes the equation of products but very little more complicated. When a polyvalent ion strikes the membrane, it will pass through only when an equivalent number of ions of opposite sign strike the membrane at the same time and pass through with it. The rate of transfer of any dissociated ionogen from one solution to the other is evidently determined by the product of all the ions required to produce the undissociated ionogen. At equilibrium, this product will have the same value in both solutions. If, for example, the system contained the ions  $\text{Na}^+$  and  $\text{SO}''_4$ , then the product  $[\text{Na}^+] \times [\text{Na}^+] \times [\text{SO}''_4]$ , or  $[\text{Na}^+]^2 \times [\text{SO}''_4]$ , would have the same value on both sides of the membrane, at equilibrium.

The impermeability of the membrane to the anion  $R'$  causes an unequal distribution of ions between the two solutions. In Solution II of the simple system including only the ionogens  $NaR$  and  $NaCl$ , let

$$x = [Na^+] = [Cl'].$$

In Solution I let

$$y = [Cl']$$

and

$$z = [R']$$

whereupon

$$[Na^+] = y + z.$$

The equation of products may then be written

$$x^2 = y(y + z).$$

But here we have the product of equals equated to the product of unequals, from which it is apparent, mathematically, that the sum of the unequals is greater than the sum of the equals, or that

$$2y + z > 2x.$$

The reasoning thus indicates that the concentration of diffusible ions in Solution I, at equilibrium, is greater than in Solution II, and this has been shown to be true in numerous experiments. If we let the excess of diffusible ions of Solution I over Solution II be represented by  $e$ , then

$$2y + z = 2x + e.$$

The different distribution of ions in the solutions at equilibrium gives rise, not only to a difference in osmotic pressure, but also to an electrical difference of potential across the membrane. Donnan derived the equation for this potential difference by the following thermodynamic reasoning.

In the system just described, let  $\pi_I$  be the potential, for positive electricity, of solution I and  $\pi_{II}$  that for Solution II. Let the extremely small quantity  $Fdn$  of positive electricity be transferred isothermally from II to I. In this virtual change of the system from equilibrium, the following work terms must be considered: the change in free electrical energy represented by  $Fdn (\pi_I - \pi_{II})$  and the simultaneous transfer of  $pdn$  moles of  $Na^+$  from II to I and of  $qdn$  moles of  $Cl'$  from I to II, where  $p$  and  $q$  are the respective transport numbers of the ions, and hence  $p + q = 1$ . The maximum osmotic work of operation of this transfer of ions is represented by the expression

$$pdnRT \cdot \log_e \frac{[Na^+]_{II}}{[Na^+]_I} + qdnRT \cdot \log_e \frac{[Cl']_I}{[Cl']_{II}}$$

But, since the system is in equilibrium, the electrical virtual work must balance the osmotic virtual work, or

$$Fdn (\pi_I - \pi_{II}) = pdnRT \cdot \log_e \frac{[Na^+]_{II}}{[Na^+]_I} + qdnRT \cdot \log_e \frac{[Cl']_I}{[Cl']_{II}}$$

But  $\frac{[Na^+]_{II}}{[Na^+]_I} = \frac{[Cl']_I}{[Cl']_{II}} = \frac{y}{x}$  and  $p + q = 1$ . Letting  $E = \pi_I - \pi_{II}$ , we have

$$E = \frac{RT}{F} \log_e \frac{y}{x} \text{ volts.}$$

This is an equation of fundamental importance in the theory of the mechanism of many reactions involved in leather making.

It will now be shown that this equation is still valid when other ions of any valency are added to the system. Consider the general case where an ionogen yielding the ion  $M^{a+}$  of valency  $a$  is added. By applying the above line of reasoning to the potential difference produced by the unequal distribution of the ions of the added ionogen between solutions I and II, we arrive at the equation

$$E = \frac{RT}{nF} \cdot \log_e \frac{[M^{a+}]_{II}}{[M^{a+}]_I}$$

where  $n = a$ , the valency of  $M^{a+}$ . But it is evident from the equation of products that

$$[M^{a+}]_I \times [Cl']^a_I = [M^{a+}]_{II} \times [Cl']^a_{II}$$

and that  $[Na^+]^a_I \times [Cl']^a_I = [Na^+]^a_{II} \times [Cl']^a_{II}$

from which it is apparent that

$$\frac{[M^{a+}]_{II}}{[M^{a+}]_I} = \frac{[Na^+]^a_{II}}{[Na^+]^a_I} = \frac{y^a}{x^a}$$

Therefore

$$E = \frac{RT}{aF} \cdot \log_e \frac{y^a}{x^a} = \frac{RT}{F} \cdot \log_e \frac{y}{x}.$$

At equilibrium, the unequal distribution of the added ionogen between solutions I and II produces exactly the same potential difference as the unequal distribution of sodium chloride. Although the addition of any ionogen must produce a change in the measured potential difference, by disturbing the equilibrium, all ionogens present when equilibrium is again established are producing the same potential difference, regardless of valency. The potential difference can thus be calculated from the determination of the distribution of only one kind of ion between the two solutions.

The complexity of systems, such as those just described, is due to the fact that the membrane prevents the diffusion of one kind of ion from one phase to the other. A similar set of conditions is brought about whenever one of a number of ions of a system is prevented from diffusing from one phase to another, which is true of every basic tannery process. When skin protein is brought into equilibrium with various tannery liquors, the diffusion of the protein ions is prevented, not by a membrane, but by their own forces of cohesion. This will be made clear in discussing the swelling of proteins.

### Swelling of Protein Jellies.

When a strip of dry gelatin is soaked in water, it swells by absorbing water, increasing in volume from 5 to 10 times, depending upon the temperature of the water and the quality of the gelatin. With increasing concentration of acid, or alkali, the swelling increases to a maximum and then decreases. The property of swelling in aqueous solutions appears to be common to all proteins under conditions such that they do not pass directly into solution. The swelling caused by acids and alkalies is generally counteracted by the addition of neutral salt or by increasing the concentration of acid or alkali sufficiently.

While attempting to arrive at a rational explanation of the molecular mechanism of tanning, Procter was continually confronted by the necessity of first explaining the mechanism of swelling and to him belongs the credit of being the first to recognize the almost complete dependence of the science of leather chemistry upon the theory of swelling. In 1897 he started an investigation<sup>54</sup> of the swelling of gelatin in solutions of acids and salts which has culminated in the Procter-Wilson theory of swelling.

Procter's general method of experimentation was as follows: Sheets of thin, purified bone gelatin were cut into portions containing exactly 1 gram each of dry gelatin. A portion was put into each of a series of stoppered bottles containing 100 cubic centimeters of hydrochloric acid of definite concentration. After 48 hours, which was shown to be sufficient for the attainment of practical equilibrium, the remaining solution was drained off and titrated with standard alkali. The gelatin plates were quickly weighed and the volume of solution absorbed was calculated from the increase in weight of the plates. The swollen gelatin was then put back into the bottles and covered with enough dry sodium chloride to saturate the solution which had been absorbed by the gelatin. This caused the gelatin to contract and give

up the absorbed solution. After 24 hours, when equilibrium was again established, the solution expelled by the salt was drained off and titrated to determine the amount of free acid which had been absorbed by the gelatin. A small amount, usually about 1 cubic centimeter, of solution always remained unexpelled by the salt and, although not strictly true, this was assumed to have the same concentration of free acid as the portion expelled, due allowance being made for the increase in volume of solution due to saturating it with salt. The acid still unaccounted for was assumed to be combined with the gelatin base.

A further set of checks was obtained by dissolving the gelatin, dehydrated by treatment with salt, in warm water and titrating with standard alkali, using both methyl orange and phenolphthalein, the former indicating the free acid left in the jelly and the latter the total, including the acid combined with the gelatin base, which was obtained by difference.

Experimental values for the volume of solution absorbed by the gelatin, the free acid left in the external solution, the free acid in the jelly, and the acid combined with the gelatin base are shown in Table XIV and in Figs. 9 and 10. These were taken from the table on page 317 of Procter's paper, *The Equilibrium of Dilute Hydrochloric Acid and Gelatin*.<sup>55</sup> In plotting the results, the concentration of gelatin chloride is taken as the difference between the concentrations of total chloride and free HCl in the jelly. The calculated values given along with the experimental ones will be discussed later in connection with the theory.

### **The Acid-Protein Equilibrium.**

Procter recognized that gelatin combines with HCl forming a highly ionizable chloride and that the resulting equilibrium is a special case of the membrane equilibria described by Donnan. Instead of tracing the development of the theory of swelling from Procter's earliest work to its present status, it will simplify matters to present the theory from the deductive reasoning furnished later by Wilson and Wilson.<sup>73</sup> They set out to prove that the entire equilibria can be determined quantitatively from the orthodox laws of physical chemistry on the simple assumption that gelatin, or any protein, combines with hydrochloric acid to form a highly ionizable chloride. It seemed that success in this would furnish substantial proof of the correctness of the theory.

In order to make the reasoning general, let us consider the hypo-



thetical protein G, which is a jelly insoluble in water, is completely permeable to water and all dissolved ionogens considered, is elastic and under all conditions under consideration follows Hooke's law, and combines chemically with the hydrogen ion, but not the anion, of the acid HA according to the equation

$$[G] \times [H^+] = K[GH^+]. \quad (1)$$

In other words, the compound GHA is completely ionized into  $GH^+$  and  $A'$ .

Now take one millimole of G and immerse it in an aqueous solution of HA. The solution penetrates G, which thereupon combines with some of the hydrogen ions, removing them from solution, and consequently the solution within the jelly will have a greater concentration of  $A'$  than of  $H^+$ , while in the external solution  $[H^+]$  is necessarily equal to  $[A']$ . The solution thus becomes separated into two phases, that within and that surrounding the jelly, and the ions of one phase must finally reach equilibrium with those of the other phase.

At equilibrium, in the external solution, let

$$x = [H^+] = [A']$$

and in the jelly phase let

$$y = [H^+]$$

and

$$z = [GH^+]$$

whence

$$[A'] = y + z.$$

It should be remembered that the brackets indicate concentration in moles per liter.

It is apparent from Donnan's line of reasoning, given earlier in the chapter, that the product  $[H^+] \times [A]$  will have the same value in the external solution as in the jelly phase at equilibrium, or that

$$x^2 = y(y + z). \quad (2)$$

As was pointed out above, it is evident from equation (2) that

$$2y + z > 2x$$

or

$$2y + z = 2x + e \quad (3)$$

where  $e$  is defined as the excess of concentration of diffusible ions of the jelly phase over that of the external solution. Where any two variables are known, all others can be calculated, for from equations (2) and (3) we get the following:

$$x = y + \sqrt{ey} = \sqrt{y^2 + yz} = (z^2 - e^2)/4e. \quad (4)$$

$$y = (-z + \sqrt{z^2 + 4x^2})/2 = (2x + e - \sqrt{4ex + e^2})/2 = (z - e)^2/4e. \quad (5)$$

$$z = (x^2 - y^2)/y = \sqrt{4ex + e^2} = e + 2\sqrt{ey}. \quad (6)$$

$$e = (x - y)^2/y = z + \frac{2y - 2\sqrt{y^2 + yz}}{\sqrt{4x^2 + z^2}} = -2x + \quad (7)$$

Since  $[A']$  is greater in the jelly than in the surrounding solution, the negative ions of the colloid compound will tend to diffuse outward into the external solution, but this they cannot do without dragging their protein cations with them. On the other hand, the cohesive forces of the elastic jelly will resist this outward pull, the quantitative measure of which is  $e$ , and according to Hooke's law

$$e = CV \quad (8)$$

where  $C$  is a constant corresponding to the bulk modulus of the protein and  $V$  is the increase in volume, in cubic centimeters, of 1 millimole of the protein.

Since we have taken 1 millimole of  $G$ ,

$$\begin{aligned} [G] + [GH^+] &= 1/(V + a) \\ \text{or } [G] &= 1/(V + a) - z \end{aligned} \quad (9)$$

where  $a$  is the initial volume of 1 millimole of the protein.

From (1) and (9)

$$z = y/(V + a)(K + y) \quad (10)$$

and from (6) and (8)

$$z = CV + 2\sqrt{CVy}. \quad (11)$$

Now from (10) and (11)

$$(V + a)(K + y)(CV + 2\sqrt{CVy}) - y = 0 \quad (12)$$

where the only variables are  $V$  and  $y$ .

If the molecules or atoms of the protein are not themselves permeable to all ions considered, the quantity  $a$  should not be taken as the whole of the initial volume of the jelly, but only as the free space within the original, dry jelly through which ions can pass. For our hypothetical protein, then, we shall consider the limiting case, where the value of  $a$  is zero. This assumption in the case of gelatin introduces errors less than the probable experimental error because of the relatively large values for  $V$  over the significant swelling range. Equation (12) thus reduces to

$$V(K + y)(CV + 2\sqrt{CVy}) - y = 0. \quad (13)$$

Knowing the values of the constants,  $K$  and  $C$ , we can plot the entire equilibrium as a function of any one variable. Procter and

Wilson<sup>56</sup> obtained the value  $K = 0.00015$  for the sample of gelatin used in their experiments by adding successive portions of standard HCl to a dilute solution of the gelatin and noting the corresponding rises in hydrogen-ion concentration. The difference between the concentration of hydrogen ion that would have been found upon adding the acid to pure water and that actually found by adding it to the same

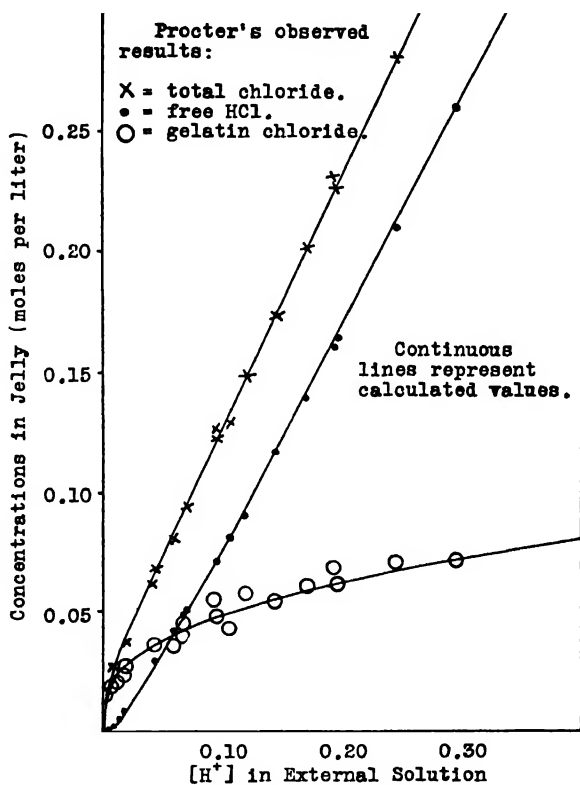


FIG. 9.—Observed and calculated values for the distribution of HCl in the system Gelatin-HCl-Water.

volume of gelatin solution was taken as the amount of acid combined with the gelatin, or as the value of  $[GH^+]$  in equation (1). Substituting any two sets of determination of  $[GH^+]$  and  $[H^+]$  in equation (1) and solving the resulting equations simultaneously, the value of  $K$  can be found.

$C$  was obtained by substituting experimental values for  $V$  and  $e$  in equation (8). It was found to vary with the temperature and with the quality of the gelatin, but had the value 0.0003 for the sample of

gelatin used by Procter and at the temperature of his experiments, 18° C.

In order to compare calculated values for V with experimental determinations of the increase in volume of 1 gram of gelatin, it is necessary to know its equivalent weight. Procter originally regarded

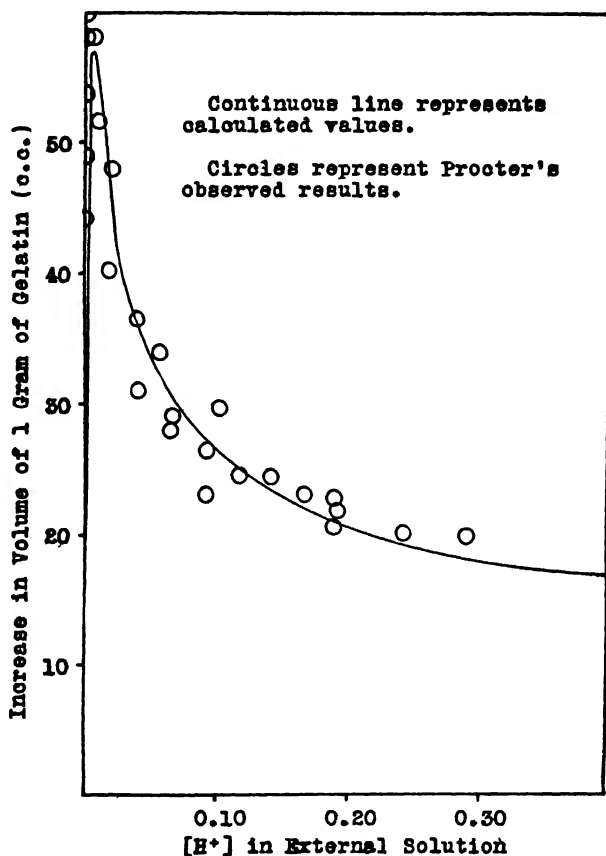


FIG. 10.—Observed and calculated values for the degree of swelling of gelatin as a function of the concentration of hydrochloric acid.

gelatin as a diacid base with a molecular weight of 839, but later work by Procter and Wilson showed that it should rather be regarded as acting as a monacid base, with an equivalent weight of 768, in acid solutions not sufficiently concentrated to cause decomposition. 768 grams of gelatin combine with a limiting value of 1 mole of hydrochloric acid and the combination resembles that of HCl with a weak monacid base. For this reason we may use the value 768 as the equiva-

lent weight of gelatin. As for the molecular weight of gelatin, no convincing figures have yet been produced and it may be questioned whether they would have any real value, if obtained. We look upon a plate of gelatin as a continuous network of chains of amino acids, there being no individual molecules, unless one wishes to look upon the plate of gelatin as one huge molecule.

From equation (13) and the values of the constants given above, Wilson and Wilson calculated all of the variables of the equilibrium for gelatin and hydrochloric acid over the range covered by Procter's experiments. The important variables are shown in Table XIV and in Figs. 9 and 10 along with Procter's actual determinations.

TABLE XIV  
AT EQUILIBRIUM.

Initial [HCl]	[HCl] in Soln.	V Calcu- lated	Cc. Solution Absorbed by 1 g. Gelatin		[HCl] in Jelly		[Total Chloride] in Jelly	
			Calcu- lated	Ob- served	Calcu- lated	Ob- served	Calcu- lated	Ob- served
0.006	0.0011	33.3	43.4	44.1	0.0001	0.0005	0.012	0.014
0.008	0.0018	37.5	48.8	48.7	0.0002	0.0004	0.014	0.015
0.010	0.0025	41.7	54.3	59.9	0.0004	0.0004	0.016	0.015
0.010	0.0028	42.7	55.6	58.4	0.0004	0.0004	0.017	0.015
0.010	0.0032	43.2	56.2	53.7	0.0005	0.0005	0.019	0.017
0.015	0.0073	40.8	53.1	57.9	0.002	0.002	0.024	0.020
0.015	0.0077	40.2	52.3	52.2	0.002	0.002	0.025	0.022
0.015	0.0120	37.5	48.8	51.9	0.005	0.006	0.031	0.027
0.020	0.0122	37.3	48.6	51.7	0.005	0.006	0.031	0.027
0.025	0.0170	34.5	44.9	40.4	0.008	0.009	0.036	0.037
0.025	0.0172	34.3	44.7	48.1	0.008	0.009	0.036	0.031
0.050	0.0406	26.7	34.8	36.4	0.026	0.030	0.063	0.061
0.050	0.0420	26.4	34.4	31.1	0.027	0.030	0.065	0.068
....	0.0576	24.0	31.2	34.0	0.041	0.043	0.082	0.079
0.075	0.0666	23.0	29.9	27.9	0.049	0.050	0.092	0.095
0.075	0.0680	22.8	29.7	29.1	0.050	0.053	0.094	0.092
0.100	0.0930	20.7	27.0	23.1	0.072	0.072	0.121	0.126
0.100	0.0944	20.5	26.7	26.4	0.073	0.072	0.122	0.121
....	0.1052	19.8	25.8	29.8	0.083	0.085	0.134	0.128
0.125	0.1180	18.9	24.6	24.4	0.095	0.090	0.148	0.148
0.150	0.1434	17.9	23.3	24.0	0.118	0.118	0.174	0.173
0.150	0.1435	17.9	23.3	24.2	0.118	0.118	0.174	0.172
0.175	0.1685	17.1	22.3	23.5	0.141	0.138	0.200	0.200
0.200	0.1925	16.3	21.2	20.6	0.164	0.161	0.225	0.229
0.200	0.1940	16.2	21.1	22.7	0.166	0.165	0.227	0.225
0.200	0.1945	16.2	21.1	22.1	0.167	0.164	0.228	0.226
0.250	0.2450	15.1	19.7	20.2	0.213	0.210	0.279	0.281
0.300	0.2950	14.0	18.2	20.0	0.261	0.260	0.332	0.332

The agreement between calculated and observed values is absolute, within the limits of experimental error. For this reason Procter and Wilson regard their theory as proved, but, if further corroboration is

desired, it can be found in the extensive researches of Loeb, some of which will be described later. *It is worthy of note that no other theory of swelling has yet passed the stage of qualitative speculation.*

Following the appearance of the work of Procter and Wilson, Hitchcock<sup>19</sup> made a number of very interesting electrometric titrations of gelatin solutions with standard hydrochloric acid solution. With each successive addition of acid, he noted the change in pH value, as in Procter and Wilson's method for the determination of the constant K. Hitchcock's value for K is 0.00024 for purified gelatin, which is of the same order of magnitude as the value 0.00015 found by Procter and Wilson.

Hitchcock tried to use his results also to calculate the equivalent weight of gelatin. Assuming that the acid disappearing from solution was combined with the gelatin, he calculated the equivalent weight of gelatin to be 1120, which is appreciably higher than the value 768 found by Procter and Wilson. However, Hitchcock's calculations assume that the solution absorbed by the particles of gelatin in the dispersion has the same concentration of free acid as that of the external solution measured by the hydrogen electrode. But the solution absorbed by the jelly particles is actually weaker in free acid than the external solution. The amount of combined acid is thus higher than calculated by Hitchcock and the value for the equivalent weight of gelatin must be taken as correspondingly less than 1120. Procter and Wilson's method was cruder, in a sense, but was not subject to this error. They titrated the acid in both external solution and in the solution extracted from the swollen jelly with salt and subtracted the quantities thus found from the total amount added to the system, which gave the amount of acid combined with the gelatin. With increasing amounts of acid used, the amount combined increased, approaching the limiting value of 1 mole of acid per 768 grams of gelatin. The author believes that 768 is nearer the true equivalent of gelatin than the higher value found by Hitchcock.

The method used both by Procter and Wilson and by Hitchcock for determining the value of K is open to the same criticism as that raised against Hitchcock's determination of equivalent weight, but it is sufficient here to have the value at least of the right order of magnitude.

Hitchcock<sup>21</sup> carried this work further in a series of experiments with deaminized gelatin. He prepared the deaminized gelatin without heating and found the product to contain less nitrogen than the original gelatin by an amount equal to 0.00040 gram equivalents of nitrogen

per gram of protein. He then proceeded to measure the acid-binding power of the deaminized gelatin as he had for the original gelatin by the method just described. The maximum combining capacity of gelatin for HCl was found to be 0.00089 gram equivalents per gram of gelatin and of deaminized gelatin only 0.00044 gram equivalents. The difference between these maximum combining capacities, 0.00045, is practically the same as the loss in nitrogen occurring in the deaminizing reaction. Since any error that may be involved in the determination of acid-combining capacities is present in both determinations, it is at least partly removed in the subtraction, making the difference correspondingly more accurate. Apparently, for each atom of nitrogen lost in the deaminizing reaction, the protein loses the capacity to combine with one hydrogen ion. This constitutes a strong confirmation of the theory that the combination of protein with acid is chemical combination. The combining capacity for HCl still retained by deaminized gelatin is presumably to be ascribed to the NH groups which are not attacked by nitrous acid.

Loebel<sup>40</sup> extended this work showing that the hydroxy groups which replace the amino groups in deaminization are acidic in character; thus deaminized gelatin has a greater power of combining with alkalis than the original gelatin by an amount equivalent to the difference in their nitrogen contents.

Gortner<sup>15</sup> made a series of studies of the acid- and alkali-binding powers of a great many proteins, using a method similar to that employed by Hitchcock. In relatively dilute solutions of acid or alkali, that is, between the pH values 2.5 and 10.5, there was evidence of chemical combination between the acid or alkali and the protein which was dependent upon the chemical composition of the protein. At higher concentrations of acid or alkali, all proteins seemed to remove equivalent quantities from solution and the logarithms of the amount of acid or alkali removed plotted against the logarithms of the original acid or alkali concentration or against the final pH value seemed to form a straight line. However, the difficulty of getting reliable measurements of this kind is very great in more concentrated solutions of acid and alkali and is complicated by hydrolysis of the protein.

Undoubtedly the simple combination of protein with acid or alkali is not the only reaction occurring in the more concentrated solutions. The simple, quantitative relations given above represent the chief factors only for dilute solutions of acids. The action of concentrated solutions of salts, as well as of acids and bases, upon proteins is not

yet well understood, although of very great importance in leather chemistry, as will be shown later. In Chapter 7 it will be shown that certain neutral salts, in concentrated solution, seem to react with collagen according to the nature of the ions involved rather than according to valency alone.

Other proteins which do not dissolve in cold water behave much

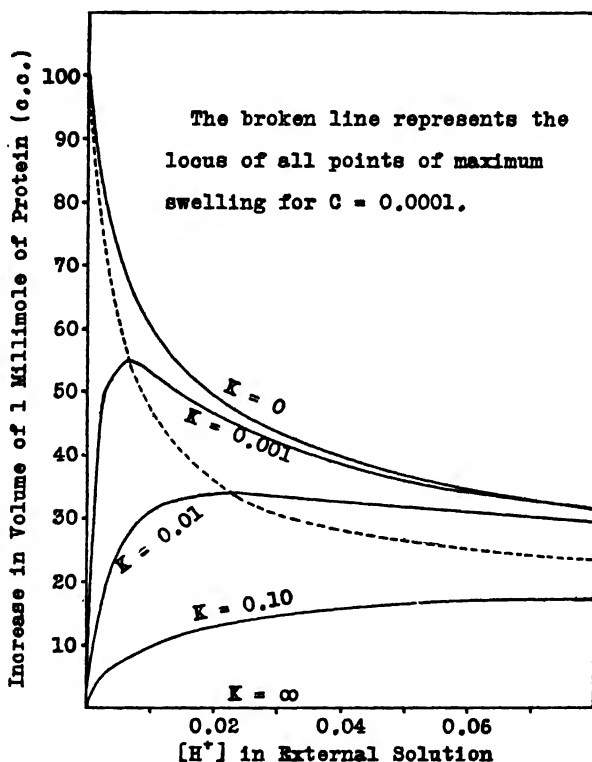


FIG. 11.—Family of swelling curves for proteins having the same bulk modulus, but different values for the hydrolysis constant.

like gelatin in respect to swelling, although they apparently have different values for the constants,  $K$  and  $C$ , as well as for equivalent weight. It is interesting to reason from the theory what differences in swelling would result from changes in the values of the constants. Since  $V = e/C$ , an increase in the value of  $C$  means a corresponding decrease in the degree of swelling. The effect of a change in the value of  $K$ , the hydrolysis constant of the protein, is shown in Fig. 11 for a fixed value of  $C$ . At  $K = 0$ , the point of maximum swelling occurs at



$x = 0$  and has the value  $I/\sqrt{C}$ . As  $K$  increases in value, the point of maximum swelling decreases in value and occurs at increasing values for  $x$ . At  $K = \infty$ , the point of maximum has the value zero and occurs at  $x = \infty$ .

### Effect of Polybasic Acids.

According to the theory, all monobasic acids should produce the same degree of swelling of gelatin for any fixed hydrogen-ion concentration, under constant conditions, provided the gelatin salts formed are practically totally ionized and provided the acid has no hydrolyzing action on the gelatin. It was formerly thought that different monobasic acids produce different degrees of swelling of gelatin, following the order known as the Hofmeister series of the ions, but Loeb and Kunitz<sup>39</sup> showed that earlier investigators, through failure to measure the hydrogen-ion concentration, had fallen into the error of attributing to the several acids effects caused merely by differences in hydrogen-ion concentration. They found practically the same degree of swelling produced by different monobasic acids for a fixed value of  $x$ , as well as by acids such as phosphoric and oxalic at concentrations at which they act as monobasic. *This was not, as many later writers seem to have supposed, a claim that the Hofmeister series is not apparent in any protein reactions, but merely proof that this series is not apparent in those phenomena which are determined entirely by the principles of the Donnan equilibrium.* Loeb has been much misquoted in this matter and criticized for claims he did not make.

The calculation of the degree of swelling of proteins in solutions of polybasic acids is not quite so simple as for monobasic acids. Suppose that  $G$  were to combine with the hydrogen ion but not the anion of the polybasic acid  $H_nA$ . Letting  $x$  represent the concentration of the polyvalent anion in the external solution at equilibrium,  $z$  the concentration of the anion of the gelatin salt, and  $y + z$  the total concentration of anion in the jelly, it is evident from the reasoning given above that

$$x^{n+1} = y^n(y + z)$$

and, by inspection of this equation, we see that

$$(n + 1)x < (n + 1)y + z$$

or that

$$(n + 1)x + e = (n + 1)y + z.$$

The total concentration of diffusible ions is greater in the jelly than in the external solution by the amount  $e$  and swelling in degree directly proportional to  $e$  will result. It can readily be seen that as  $x$  increases from zero, without limit,  $e$  and the degree of swelling increase to a maximum and then decrease, approaching zero, for  $z$  has a limiting value since it cannot exceed the total concentration of gelatin. At  $x = 0$ ,  $y = 0$ , and  $e = 0$ . As  $x$  increases without limit, our equations approach the limiting relations

$$x^{a+1} = y^{a+1}$$

and

$$(a + 1)x + c = (a + 1)y$$

from which it is evident that  $x = y$  and  $e = 0$ .

The extent of swelling by polybasic acids which combine as such with the protein will be considerably less than that caused by monobasic acids, as Loeb has shown, because fewer anions will be associated with equivalent weights of the protein. For example, for equivalent weights of gelatin sulfate and gelatin chloride, there would be only half as many sulfate ions as chloride ions. For very small values of  $x$ , we should therefore expect sulfuric acid to produce only half as much swelling as hydrochloric acid at the same hydrogen-ion concentration and this is actually the case.

### Repression of Swelling by Salts.

The theory accounts quantitatively for the action of neutral salts in repressing the swelling of proteins by acid. In the system described above in which the protein G was immersed in a solution of HA, consider the addition of the mono-monovalent salt MN, neither of whose ions combine with G. At equilibrium, let the concentration of  $M^+$  be represented by  $u$  in the external solution and by  $v$  in the jelly. It is evident from the general equation of products that the product

$$([H^+] + [M^+]) \times ([A'] + [N'])$$

will have the same value in both phases, or that

$$(x + u)^2 = (y + v)(y + v + z)$$

from which

$$e = 2(y + v) + z - 2(x + u).$$

Solving the two preceding equations simultaneously, we get

$$e = -2(x + u) + \sqrt{4(x + u)^2 + z^2}.$$

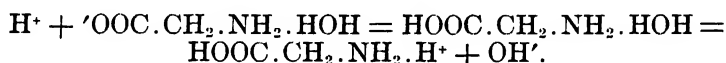
Now, if the value of  $x + u$  increases while  $z$  remains constant, the value of  $e$ , and consequently the swelling, will decrease. The addition

of MN to the system increases  $u$  and hence must cause a decrease in the degree of swelling, since it increases  $z$  only by causing a diminution of the volume of the jelly.

It is important to recognize that the repression of swelling by salts does not depend upon any repression of ionization of the protein salt. The salt acts so as to lower the value of  $e$ , which is the measure of the force producing swelling. In some cases, the ionization may be repressed to some extent and this would assist in repressing the swelling, but in the case of gelatin chloride, the swelling is markedly reduced long before there is any repression of ionization of gelatin chloride measurable by means of calomel electrodes.

### The Alkali-Protein Equilibrium.

Proteins are amphoteric substances, reacting both as weak acids and as weak bases. In this respect, they retain the properties of the amino acids from which they are formed. Hydrated aminoacetic acid is capable of assuming either a positive or negative charge, or both, by ionizing as acid or base, or both, thus:



The ionization constant of a protein as an acid may be represented as follows:

$$[\text{H}^+] \times [\text{G}'] = K_a[\text{GH}].$$

But  $[\text{H}^+] \times [\text{OH}'] = K_w$  or  $[\text{H}^+] = K_w/[\text{OH}']$

from which  $[\text{GH}] \times [\text{OH}'] = k[\text{G}']$ , where  $k = K_w/K_a$ .

But this is essentially the same as equation (1) except for the fact that  $[\text{H}^+]$  is replaced by  $[\text{OH}']$ . It is thus apparent that proteins will behave in solutions of increasing concentration of alkali much as they do in solutions of acids so long as they undergo no chemical changes other than that of salt formation. Actually gelatin swells in alkaline solution to a maximum at a concentration of about 0.004 mole of hydroxide ion per liter, above which the swelling diminishes. In acid solution maximum swelling occurs at a concentration of 0.004 mole of hydrogen ion per liter.

The effect of valency is similar in both acid and alkaline solutions. Loeb found that the diacid bases calcium hydroxide and barium hydroxide give points of maximum swelling for gelatin only half as great as the monacid bases. For a given pH value, the amount of swelling is determined by the valency of the ions of opposite sign to

that of the protein ions rather than by the specific nature of the ions themselves.

In alkaline solution the protein ion is negatively charged, while it is positively charged in acid solution. In a solution, originally alkaline, in which the hydrogen-ion concentration is gradually increased, there must be some point at which the protein becomes electrically neutral; that is, where it has an equivalent number of positive and negative charges. The hydrogen-ion concentration at which this occurs has been called by Hardy<sup>16</sup> the isoelectric point of the protein. The isoelectric point of gelatin was found by Michaelis and Grineff<sup>47</sup> to lie at a pH value of 4.7 and this value has been repeatedly confirmed by Loeb and others.

According to the chemical theory of the combination of acids with gelatin, the removal of basic nitrogen groups from the gelatin molecule should shift the isoelectric point to lower values and this is exactly what Hitchcock<sup>21</sup> found; deaminization of gelatin caused its isoelectric point to drop from pH = 4.7 to pH = 4.0.

Thomas and Kelly<sup>67</sup> determined the isoelectric point of collagen, or rather hide powder, by means of acid and basic dyes. Portions of hide powder were first wet with solutions of different pH values, then with solutions of basic fuchsin or Martius yellow, and finally washed with solutions having the same pH values as were used to wet the portions initially. The fuchsin left the hide powder deeply stained only at pH values greater than 5 and the Martius yellow only at values below 5, indicating pH = 5 as the isoelectric point of collagen.

Porter<sup>52</sup> observed that a point of minimum swelling of hide powder occurs at a pH value of 4.8, indicating this as its isoelectric point. Porter also found points of maximum swelling of hide powder at pH values of 2.4 in acid solution and about 12.3 in alkaline solution.

Thomas and Kelly compiled a list of isoelectric points of different proteins, taken from the literature, and these have been added to Table XV in terms of pH value.

### **Two Forms of Collagen and Gelatin.**

Quantitative experiments upon alkaline swelling are rendered difficult by the tendency for the gelatin to pass into solution, which is very much more marked than for acid swollen gelatin. That gelatin and some other proteins undergo a change of form in alkaline solutions is apparent from recent experimental data. Lloyd<sup>28</sup> observed a rather significant change occurring in gelatin dissolved in alkaline solution.

TABLE XV.

ISOELECTRIC POINTS OF SOME PROTEINS IN TERMS OF pH VALUE.

Protein	pH Value	References
Collagen .....	5.0	52, 67
	5.1, 7.6	71
Deaminized collagen .....	4.0, 8.3	64
Gelatin .....	4.7	28, 47
	4.7, 7.7	18, 72
Deaminized gelatin .....	4.0	21
	4.0, 7.3	40
Wool .....	3.5	44
Silk fibroin .....	4.2	44
Serum albumin .....	4.7	46
Serum globulin .....	5.4	58
Egg albumin (hen) .....	4.8	63
Denatured serum albumin .....	5.4	46
Oxyhemoglobin .....	6.7	50
Carbon monoxide hemoglobin .....	6.8	45
Reduced hemoglobin .....	6.8	45
Stroma globulins of blood corpuscles .....	5.0	46, 50
Red blood cells .....	4.6	7
Yeast extract proteins (globulins) .....	4.6	12
Gliadin .....	9.2	58
Edestin .....	5.6	48
Tuberin (potato) .....	4.0	6
Carrot protein .....	4.0	6
Tomato protein .....	5.0	6
Nucleic acid .....	2.0	46

A comparison between gelatin dissolved in acid solution and gelatin dissolved in alkaline solution was made as follows.

Two grams of gelatin were put into a flask containing 200 cubic centimeters of tenth-molar hydrochloric acid. After 6 days at 20° C., the gelatin was completely dissolved and 20 cubic centimeters of molar sodium hydroxide were added to the solution, which was then tested and found to be neutral to litmus. 220 cubic centimeters of saturated ammonium sulfate solution were then added and a white, flocculent precipitate formed, which was filtered off. The filtrate was tested and found to be free from protein. The precipitate was insoluble in cold water and was washed several times. It was dissolved in 2 cubic centimeters of hot water and set to a jelly upon cooling. A control experiment made by dissolving 2 grams of gelatin in 220 cubic centimeters of water with 1.12 grams of sodium chloride behaved in a similar manner.

For comparison, 0.2 gram of gelatin were put into a flask containing 200 cubic centimeters of tenth-molar sodium hydroxide. The gelatin was completely dissolved after 2 days at 20° C. 20 cubic centimeters of molar hydrochloric acid were then added to the solution,

after which it reacted neutral to litmus. 220 cubic centimeters of saturated ammonium sulfate solution were added and a white, flocculent precipitate formed, which was filtered off. The filtrate, as in the previous experiment, was found to be free from protein. But the precipitate dissolved completely and rapidly in a small volume of cold water and would not set to a jelly even when the volume was reduced to 2 cubic centimeters.

Lloyd suggested that gelatin changes from a keto-form to an enol-form in alkaline solution. The gelatin recovered from acid solution and which had the power of setting to a jelly would thus be regarded as the keto-form of gelatin, while that recovered from alkaline solution and which had lost the power of setting to a jelly would be looked upon as the enol-form of gelatin. Miss Lloyd regarded the change in alkaline solution as irreversible, but her experiments do not show this. Mr. Kern, in the author's laboratory, added hydrochloric acid to gelatin dissolved in a hot solution of sodium hydroxide until the pH value, as determined by the hydrogen electrode, was reduced to 4.7 and then allowed the solution to cool, whereupon it set to a firm jelly, indicating that the change is reversible. Miss Lloyd's experiment showed merely that it is not readily reversed by the addition only of the quantity of hydrochloric acid equivalent to that of the sodium hydroxide originally employed.

In studying the degree of plumping of calf skin as a function of pH value, Wilson and Gallun<sup>71</sup> found two points of minimum, one at 5.1 and the other at 7.6. This work will be described in Chapter 10. Wilson and Kern<sup>72</sup> followed this with a series of experiments upon the swelling of gelatin in buffer solutions and also found two points of minimum, one at 4.7 and the other at 7.7. A description of their work follows.

A series of buffer solutions was prepared, each member of which had a final concentration of tenth-molar phosphoric acid plus the amount of sodium hydroxide required to give the desired pH value as determined by the hydrogen electrode at 20° C. The pH values ranged from 3 to 12. 200 cubic centimeters of each solution were put into a stoppered bottle and kept in a thermostat refrigerator at 7° C. After the temperature of each solution had reached 7°, a small strip of high grade gelatin of known weight was put into it. All strips were taken as nearly alike as possible and were kept in the solutions at 7° for 4 days, after which each strip was quickly blotted off and weighed. The results were carefully rechecked. In Table XVI are given the gain in weight per gram of dry gelatin and the initial and final pH

TABLE XVI.

SWELLING OF GELATIN IN PHOSPHATE BUFFER SOLUTION DURING 4 DAYS AT 7° C.

pH Value of Buffer Solution at 20° C.		Increase in Wt. of 1 g. Dry Gelatin Grams
Initial	Final	
2.90	2.92	13.20
3.50	3.50	9.49
3.96	4.01	7.72
4.14	4.17	6.91
4.47	4.59	6.68
4.78	4.86	6.20
5.08	5.12	7.02
5.29	5.38	7.13
5.57	5.61	7.22
5.78	5.80	7.56
6.04	6.08	7.80
6.29	6.29	7.83
6.48	6.49	8.02
6.69	6.70	8.29
6.96	6.94	8.31
7.08	7.10	8.25
7.41	7.37	8.03
7.68	7.62	7.62
7.97	7.89	8.39
8.42	8.36	8.59
8.56	8.48	8.60
9.03	8.96	8.78
9.57	9.51	8.91
10.00	9.96	8.98
10.47	10.41	9.24
11.06	10.98	9.55
11.52	11.48	9.95
12.00	11.95	10.73

values of the buffer solutions. Fig. 12 represents the degree of swelling as a function of the pH value.

Wilson and Kern suggested that the two points of minimum represent the isoelectric points of the two forms of gelatin described by Lloyd, and this view appears to be substantiated by other data available in the literature.

Because of the apparent importance of the finding and of the paucity of confirming data, questions were raised as to whether the finding was not due to some impurity in the particular sample of gelatin used or to the buffer solution. Dr. Sheppard furnished a sample of ash-free, isoelectric gelatin, prepared according to the method of Sheppard, Sweet, and Benedict,<sup>61</sup> with which Wilson and Kern<sup>72</sup> repeated their experiment, using an even more dilute buffer solution and checked their previous finding exactly.

It was then pointed out that the phosphate in the buffer solution introduced a variable factor that might account for the finding. At-

tempts to repeat the work without the use of buffers were unsatisfactory because of the difficulty of bringing the system to equilibrium at pH values in the neighborhood of 8. But confirmation of the finding of Wilson and Kern, without the use of buffers, came from an unexpected source. Higley and Mathews<sup>18</sup> measured the absorption of light by gelatin solutions as a function of pH value, using a spectro-

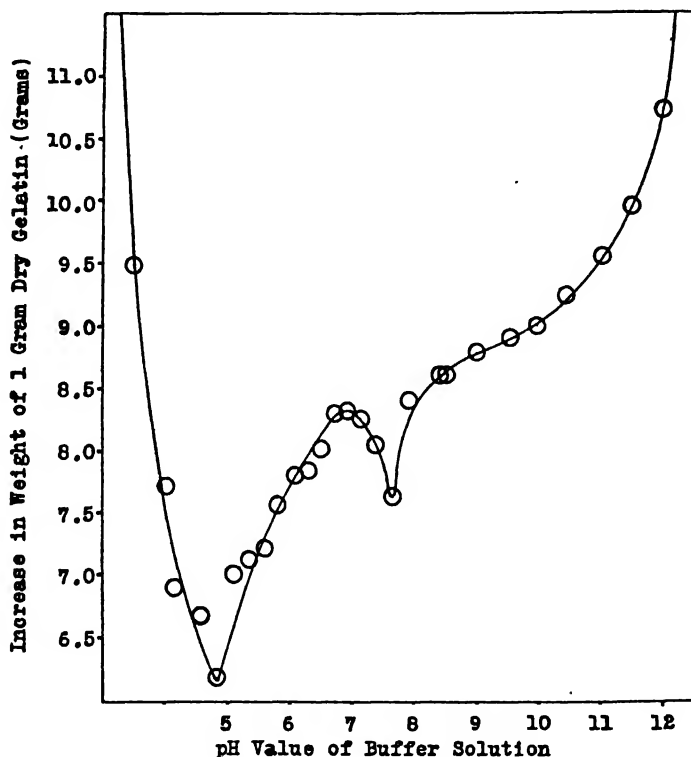


FIG. 12.—Showing the two points of minimum swelling of gelatin.

photometric method. They varied the pH value by adding hydrochloric acid or sodium hydroxide to solutions of highly purified gelatin in 1 per cent final concentration. Measurements of pH value were made with the hydrogen electrode. The wave lengths for definite fractions of light absorbed are plotted in Fig. 13. The fractions are 0.6, 0.4, 0.2, and 0 (transparent). The sharp shift of the absorption band toward the ultraviolet as the pH values 4.7 and 7.7 are approached strikingly resembles the minimum regions in the swelling curve of Wilson and Kern.



Experiments upon the mutarotation of gelatin led Smith<sup>62</sup> to suggest that gelatin exists in two forms: a sol form, having a specific rotation of  $[\alpha]_D = -141$  and being stable at temperatures above  $35^\circ \text{C.}$ , and a gel form, with a specific rotation of  $[\alpha]_D = -313$  and stable under  $15^\circ$ , a condition of equilibrium existing between the two forms at intermediate temperatures. The gel form is characterized by

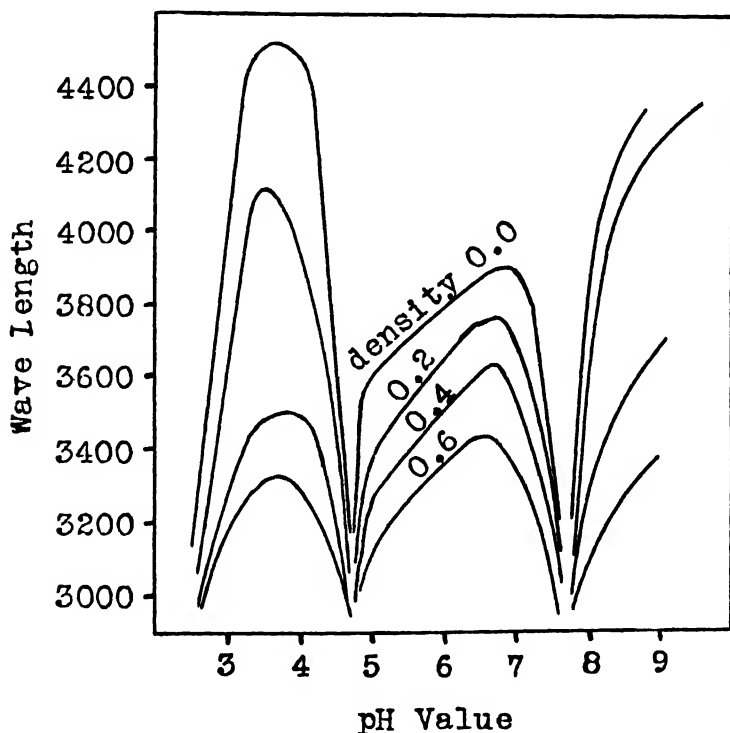


Fig. 13.—Wave lengths for definite fractions of light absorbed by gelatin solution as functions of pH value.

its power to set to a jelly, which is lacking in the sol form. Smith calculated that a concentration of from 0.6 to 1.0 gram of the gel form per 100 cubic centimeters is required to produce gelation. As the temperature is increased above  $15^\circ$ , the total concentration of gelatin required to produce gelation is increased because of the decreasing proportion of the gel form, which does not exist at all above  $35^\circ \text{C.}$  Gelatin is the only protein known to show mutarotation, but it gradually loses this property along with its jellying power, when its solutions are kept at temperatures above  $70^\circ \text{C.}$

Davis and Oakes<sup>8</sup> measured the viscosities of a series of solutions of gelatin at 40° C. at different pH values. Their results, which are reproduced in Fig. 14, indicate a point of minimum at about 8, but none at 4.7. If the theory of Wilson and Kern were valid, the finding

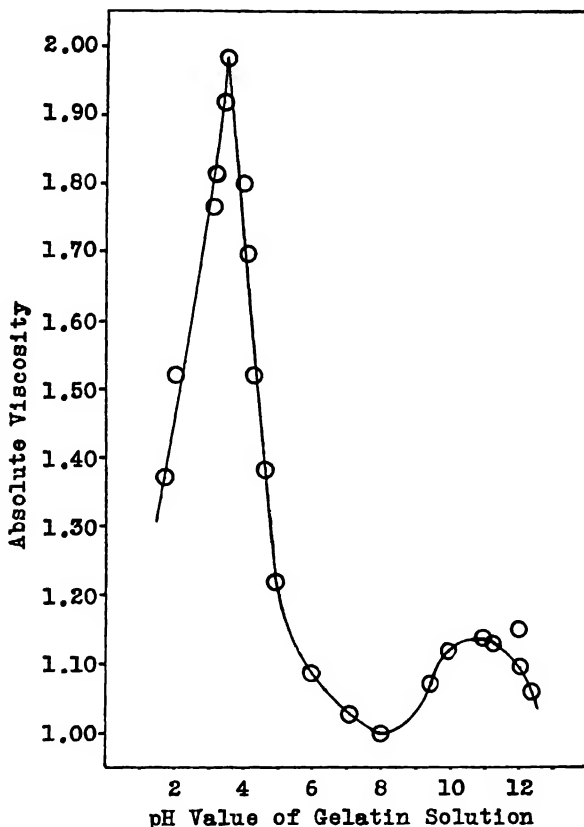


FIG. 14.—Variation of viscosity of 1-per cent solution of gelatin at 40° C. with change of pH value (Davis and Oakes).

of Davis and Oakes would be easily explainable as indicating the isoelectric point of the sol form of gelatin.

But Hitchcock<sup>22</sup> measured the viscosity of gelatin solutions at 40° C. and found points of minimum only at pH = 4.7. His results are given in Fig. 15. He suggested that the different shape of the curve obtained by Davis and Oakes may have been due to their method of preparing the solutions, which included heating the gelatin to 75° C. It is possible that complete conversion of the gel to the sol

form of gelatin required the drastic treatment given by Davis and Oakes. Since no values were obtained by Hitchcock for pH values between 7 and 8, his curves tell nothing about the so-called second point of minimum.

Another case of the apparent disappearance of the point of minimum at pH = 4.7 when working at a temperature of 40° C. is to be

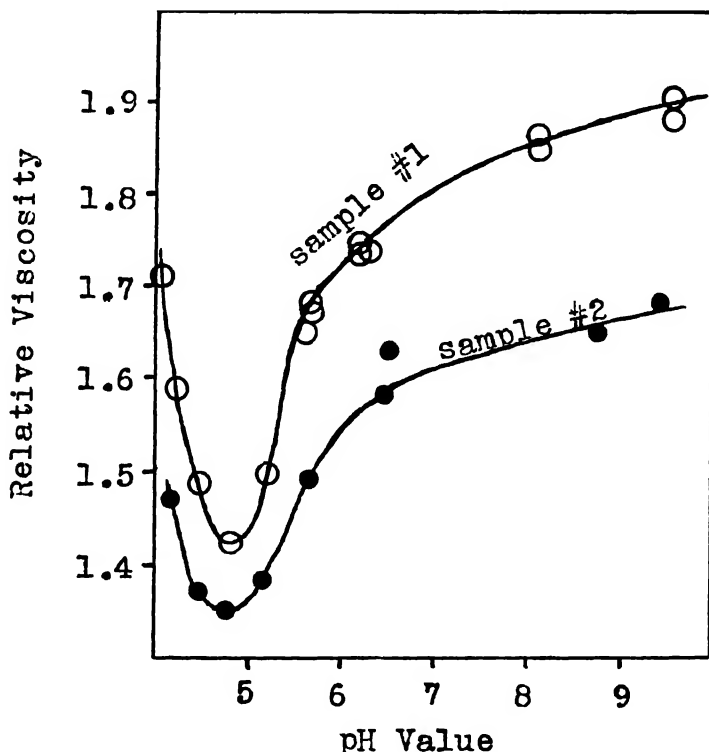


FIG. 15.—Variation of viscosity of 1-per cent solutions of gelatin at 40° C. with change of pH value (Hitchcock).

found in the work of Wilson and Daub,<sup>70</sup> who experimented upon the bating of calf skin at 40° at different pH values. They observed a point of minimum plumping only in the region of pH = 8, but this may have been due to the increased activity of trypsin at this point.

Wilson and Gallun<sup>71</sup> observed points of minimum plumping of bated calf skin at both pH = 5 and pH = 8, when working at low temperatures. The recent work of Sheppard, Sweet and Benedict<sup>61</sup> adds further evidence of the existence of critical pH values at both 5 and 8. They obtained a curve for the rigidity of gelatin jelly as a

function of pH value exhibiting a shoulder at 5 and a maximum between 7 and 9.

Apparently the change in gelatin from the gel form to what has been called the sol form takes place both with rise of temperature and with rise of pH value. Since the experiments of Wilson and Kern were performed at 7° C., they were dealing with the gel form of gelatin in acid solution and actually observed a point of minimum at  $\text{pH} = 4.7$ , the isoelectric point of the gel form. The appearance of a second point of minimum swelling at  $\text{pH} = 7.7$  seems to indicate that between 4.7 and 7.7 the gelatin passes from the gel to the sol form and that the second point of minimum occurs at the isoelectric point of the sol form. It was only by working at temperatures as low as 7° that they were able to prevent the gelatin from passing into solution at the higher pH values.

While objection may be raised to the terms gel and sol form as applied to the two forms of gelatin and of collagen, they will serve as well as any until more is known of the transition. Lloyd's suggestion that the change is a keto-enol tautomerism is still speculative, although not incompatible with the theory of the dioxopiperazine structure of the proteins, described in Chapter 3.

Thomas and his coworkers have given further evidence in support of the theory that gelatin and collagen undergo an important change in molecular structure as the pH value of the accompanying solution is raised from 4.7. This will be discussed in connection with theories of tanning in Volume II.

### **Electrical Potential Difference between Protein Jelly and Aqueous Solution.**

It is apparent from the discussion of Donnan's theory of membrane equilibria that the unequal distribution of ions between a jelly and its surrounding solution must give rise to an electrical difference of potential between these two phases whose measure is  $(RT/F) \cdot \log(x/y)$ , where  $x$  is the hydrogen-ion concentration of the external solution and  $y$  that of the solution within the jelly and this value holds true regardless of the valence or number of ions in the system. The potential difference can therefore be calculated from the determinations of pH value in the jelly and in the external solution. Changing from natural to common logarithms and substituting the numerical value for  $RT/F$  at 20° C., we get

$$\text{P.D.} = 58 \log(x/y) = 58(\log x - \log y) \text{ millivolts.}$$

But  $-\log y = \text{pH value of the jelly}$  and  $+\log x = -\text{pH value of the solution}$ . Hence, at  $20^\circ \text{C}$ ,

$$\text{P.D.} = 58(\text{pH of jelly minus pH of solution}) \text{ millivolts.}$$

Loeb<sup>88</sup> devised a very ingenious method for determining this potential difference directly by means of a pair of calomel cells of equal value and a Compton electrometer. A diagram of his apparatus is shown in Fig. 16. The potential difference measured is that of the cell

calomel electrode	saturated KCl	external solution	solid jelly	saturated KCl	calomel electrode
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Everything else being symmetrical, the potential difference measured is that between the jelly and the external solution with which it is supposed to be at equilibrium.

In a typical experiment, 1 gram of purified gelatin, powdered to a grain size between 30 and 60 mesh, was put into each of a series of solutions of different concentrations of hydrochloric acid or sodium

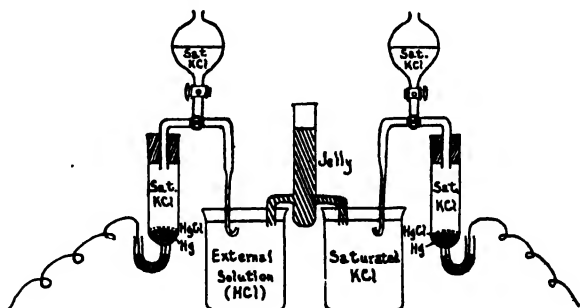


FIG. 16.—Loeb's apparatus for measuring the potential difference between gelatin jelly and the surrounding solution.

hydroxide. The volume of each solution was 350 cubic centimeters and the temperature  $20^\circ \text{C}$ . After 4 hours the volume occupied by each portion of gelatin was measured, the solution filtered off, and the gelatin melted so that the pH values of both jelly and solution could be determined by means of the hydrogen electrode. The gelatin was then allowed to set to a jelly in the receptacle illustrated in Fig. 16 and the potential difference between the jelly and external solution was then measured with a Compton electrometer. The results of such a series are shown in Table XVII along with calculations of the potential differences made from the pH determinations. The calculated and observed results are at least of the same sign and order of

TABLE XVII.

## SUSPENSIONS OF POWDERED GELATIN.

After 4 Hours at 20° C.							
Initial Normality of Solution	Vol. of Gelatin (mm.)	pH Value of		(a) Minus (b)	P.D. Millivolts		
		Absorbed	External		Calcu- lated	Observed	
		Solution (a)	Solution (b)				
0.0010N HCl .....	28	4.44	3.35	+ 1.09	+ 63.0	+ 56.0	
0.0005N HCl .....	20	4.56	3.55	+ 1.01	+ 58.6	+ 55.5	
0.0002N HCl .....	18	4.79	3.92	+ 0.87	+ 51.0	+ 36.5	
0.0001N HCl .....	16	4.85	4.24	+ 0.61	+ 36.0	+ 15.0	
Water .....	17	4.89	4.97	— 0.08	— 4.5	— 17.5	
0.0001N NaOH .....	18	4.98	5.96	— 0.98	— 57.0	— 59.0	
0.0002N NaOH .....	28	5.06	6.24	— 1.18	— 68.0	— 61.0	
0.0005N NaOH .....	37	5.50	6.46	— 0.96	— 56.0	— 70.0	
0.0010N NaOH .....	40	6.74	7.30	— 0.56	— 33.0	— 66.0	
0.0020N NaOH .....	47	9.54	10.56	— 1.02	— 59.0	— 46.0	
0.0040N NaOH .....	48	10.15	11.08	— 0.93	— 48.0	— 36.0	

magnitude, which is a good agreement considering the nature of the experiments and the dilutions of the solutions. It will be shown later that the method is capable of very much better agreement where the complications involved in melting and resetting of the jelly are avoided, as in the measurement of potential difference between a solution of gelatin and a protein-free solution with which it is in equilibrium and from which it is separated by a semi-permeable membrane, especially where the solutions have greater conductivities.

According to the theory, the concentration of free acid in an acid-swollen jelly should be less than that in the external solution and, likewise, the concentration of free alkali in an alkali-swollen jelly should be less than that in the external solution with which it is in equilibrium. This is verified by the figures in Table XVII, which show, for pH values of the external solution less than 4.7, that the hydrogen-ion concentration is greater in the solution than in the jelly, while for pH values of the external solution greater than 4.7, the hydrogen-ion concentration is less or the hydroxide-ion concentration greater in the solution than in the jelly.

### Rhythmic Swelling of Protein Jellies.

Sheppard and Elliott<sup>59</sup> made a study of the causes of the reticulation of the surfaces of photographic negatives that has a bearing upon a similar kind of trouble sometimes occurring in the vegetable tanning of skins. During the fixing or washing of a negative, the wet gelatin layer sometimes becomes more or less finely wrinkled or corrugated,

the network of puckers forming a pattern extending either over the whole of the negative or only over part of it. They found that this reticulation can be produced by the combined action of a swelling agent and a tanning agent.

Fig. 17 represents a print from a negative treated to produce reticulation by Mr. Daub in the author's laboratories. The plate was

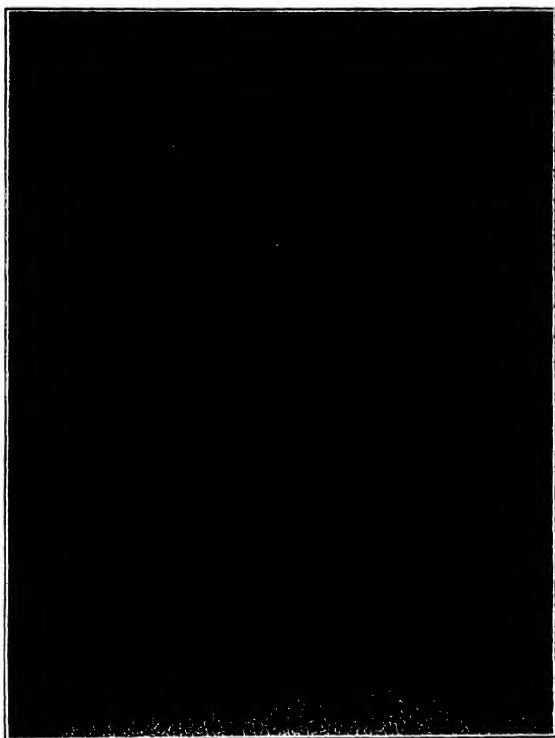


FIG. 17.—Reticulation produced on photographic negative.

flashed, developed, fixed with sodium thiosulfate, washed, and then immersed in a solution of wattle bark extract containing 5 grams of tannin and 0.2 mole of acetic acid per liter; the temperature was kept at 28° C. After several minutes the gelatin surface began to pucker at isolated points and this action gradually spread over the entire surface, producing series of ridges of swollen gelatin with valleys of hardened and contracted gelatin in between. Following this action, the silver particles migrated from the hardening portions into the swelling ridges, giving the negative the mosaic-like appearance shown

in the print. Often the puckering became well pronounced before the migration of silver particles was noticeable. Sheppard and Elliott liken the effect to the production of Liesegang rings.

The acid tends to cause a swelling of the gelatin while the tannin tends to cause a hardening and contracting action. But the acid diffuses relatively very rapidly, whereas the diffusion of the tannin is greatly retarded both by its high molecular weight and by its tendency to combine with the gelatin, forming a compound less permeable and having a much lower power of swelling than the original gelatin. The action becomes greatly accelerated as the temperature is raised towards the melting point of the gelatin jelly. When the action is prolonged at higher temperatures, provided the jelly does not dissolve, a second and much coarser series of puckers begins to form, tending to mask the finer pattern. In the coarser pattern, the peaks of the ridges may be from one to several millimeters apart.

The reticulation of the surface of skin in tanning is a very serious matter as the pattern formed is permanent and materially reduces the selling value of the leather. The pattern formed on skins is usually of the coarser variety and would hardly pass as an artistic sample of embossing, which the photographic negative might do, because of the fineness of the pattern and distribution of silver particles. The reticulation of skin may attend the injudicious use of acid in attempting to plump the leather during tanning, or it may occur where acid-producing ferments get the upper hand in a yard where fresh liquors are normally used. The corrective is to prevent the swelling action, either by neutralization of the acid or by the addition of salt.

### Structure of Gelatin Solutions and Jellies.

Procter's<sup>53</sup> investigations of the behavior of gelatin jellies led him to regard them as having a structure consisting of a network of molecules cohering to each other, but leaving interstices large enough to permit the passage of water and simple molecules and ions. The long chains of amino acids making up the protein molecules are peculiarly fitted to produce such a structure through combination of the acid and basic terminals of these chains. A hot solution of gelatin may be looked upon as a true solution consisting of individual gelatin molecules, or at least of comparatively small polymerized groups, but the molecules orientate themselves, as the solution cools, so as to leave a minimum of free energy, the most active acid groups tending to unite with the most active basic groups until a continuous network



is formed throughout the system. A block of jelly might thus be looked upon as an enormous, single molecule. Such a view is not radical in the light of modern theories of crystal structure.

According to the Procter-Wilson theory of swelling, when a block of gelatin jelly is immersed in a solution of hydrochloric acid, the solution passes into the jelly, filling up the interstices. Of the ionized gelatin chloride, which then forms, the chloride ions remain in the solution in the interstices while their corresponding gelatin cations form part of the network and are not in solution in the same sense as the anions. In tending to diffuse into the outer solution, the anions exert a pull upon the cations forming part of the network, causing an increase in volume of the jelly proportional to the pull exerted, so long as the elastic limit is not exceeded. That gelatin jellies are truly elastic and follow Hooke's law may be taken as proved chemically by the agreement between calculated and observed results shown in Table XIV. More recently Sheppard and Sweet<sup>60</sup> proved by measurements of rigidity that gelatin jellies follow Hooke's law nearly up to the breaking point.

The modulus of elasticity of a sample of gelatin jelly is evidently determined by its inner structure, but the nature of this structure apparently depends upon the concentration of the gelatin at the moment of setting. A dilute solution would set with the interstices of the molecular network more voluminous than in the case of a concentrated solution. If both jellies were later dried out to the same extent, they might look alike, but would differ in inner structure; when placed in water, the one formed in the more dilute solution should swell to a greater extent than the other. This is exactly what Procter found. He prepared three solutions containing 5 per cent, 10 per cent, and 20 per cent, respectively, of gelatin and allowed them to set. He then dried the jellies and, after weighing, allowed them to soak in water for 7 days. The sample with the greatest volume at time of setting absorbed 14.6 times its weight of water, the second sample 7.7, and the sample with the smallest setting volume only 5.8 times its weight of water.

As Sheppard and Elliott have shown, when a gelatin solution is allowed to set in a mold and then dried, it loses entirely the shape of the mold, as shown in Plates 64, 65, 66 and 67 of Chapter 8, where their work is described. But when soaked again in water, the gelatin swells so as to regain its lost shape. A thin sheet of jelly adhering to a glass plate, upon drying, shrinks in thickness to a very much greater degree than in either of the other two dimensions. Upon

soaking the dried sheet in water, it swells chiefly in thickness, tending to assume its shape at the time of setting.

Loeb's work on the viscosity of gelatin solutions, to be discussed presently, indicates that the initial step in gelation is the combination of individual molecules to form large aggregates, possibly in a manner similar to the growth of crystals. Bogue<sup>2</sup> pictures this process as the formation of catenary threads by the union of the individual molecules end to end. The manner in which fibrous curds of soap are formed led McBain<sup>42</sup> to a similar view regarding the structure of soap jellies and solutions. He attributes the elasticity of gels to the formation of an exceedingly fine filamentous structure. Innumerable molecules placed lengthwise and held together by forces of residual valence are assumed to make up these fine threads, which may be microns or millimeters in length.

Considering the nature and variety of the amino acids composing the gelatin molecule, as shown in Table I of Chapter 3, we should hardly expect the polymerization of gelatin to take place along a single line, but in every direction and probably with cross chains growing to support chains increasing in length in other directions. The increasing viscosity of gelatin solutions with time, upon cooling, would thus be attributed to the increasing size of the particles; the formation of a rigid jelly to the final union of the large particles, forming a structure continuous throughout the entire system.

There is an abundance of evidence to support Procter's view of the structure of jellies and Loeb's view that gelatin solutions, after standing for a time at temperatures below 35° C., always contain particles of jelly consisting of aggregates of gelatin molecules. A number of supporting lines of evidence are given in a review of the literature by Thompson.<sup>68</sup>

Graham showed long ago that the velocity with which crystalloids diffuse through gelatin jellies is only very little less than the velocity through pure water. This slight reduction in velocity is in no way comparable with the apparently great physical difference in state between the jelly and water. Although the viscosity of a gelatin jelly is too great to be measured by the methods usually applied to liquids, simple molecules move through it as though in a medium of viscosity nearly that of water. The network theory explains this by assuming that the diffusing substance actually is moving through the pure water or aqueous solution in the interstices of the network. Any slight diminution in velocity can be accounted for by the small portion of any cross section of the jelly occupied by the gelatin network. The

same holds true for gelatin solutions, the diffusing substance being able to pass through the particles of jelly in suspension almost as rapidly as through the solution surrounding the particles.

Thompson shows from the work of Dumanski<sup>11</sup> that the conductivity of a solution of potassium chloride in gelatin jelly is no less than in pure water when a correction is made for the small volume actually occupied by the gelatin network, whereas, if the apparent viscosity had any effect, the conductivity should be reduced by the gelatin to a minute fraction of its value in pure water.

The vapor pressure of even a 20-per cent gelatin jelly is practically the same as that of water, indicating the presence of pure water in accordance with the network theory.

By placing a strain upon gelatin jelly in one direction, double refraction is produced, a property always associated with a definite structure and with anisotropy. Even dilute solutions of gelatin show double refraction on compression or when passed between two cylinders rotating in opposite directions. With increasing strain, the effect is increased up to a point corresponding to an elastic limit. This indication of structure even in gelatin solutions corroborates the views of Loeb and of Bogue.

The fact that the viscosity of gelatin solutions is lowered by simply agitating the solution is another piece of evidence in favor of the existence of a structure in gelatin solutions and still further evidence is furnished by Loeb's work on the viscosity of gelatin solutions and Bogue's measurements of plasticity, to be described later.

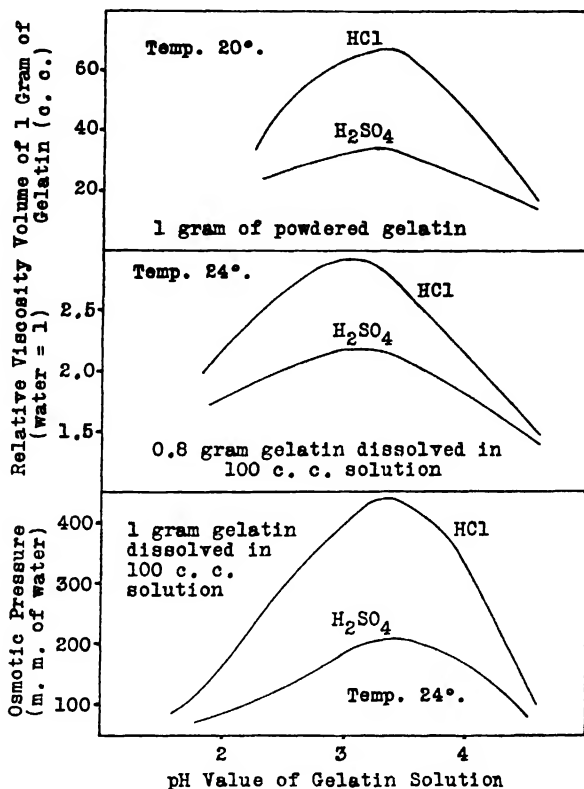
### Relation of the Osmotic Pressure and Viscosity of Gelatin Solutions to the Swelling of Gelatin Jellies.

In an extensive series of experiments, Loeb has shown that the variations in osmotic pressure and viscosity of gelatin solutions with change of pH value or of concentration of salt, parallel the corresponding variations in the degree of swelling of gelatin jellies, which is what would be expected on the basis of the theory of protein-salt formation described above. This parallelism is shown by the curves in Figs. 18 to 23.

In each determination<sup>30</sup> of the two series of experiments performed to get the curves shown in Fig. 18, 1 gram of powdered gelatin was put for 1 hour at 20° C. into 100 cubic centimeters of acid solution of definite strength. The volume of the gelatin was measured, after

settling, in a graduated cylinder and the pH value of the jelly was determined after melting. The volume is plotted against the pH value of the jelly and not that of the external solution, which was always lower, as explained in the discussion of the theory of swelling.

The curves in Fig. 19 were obtained by rapidly heating to 45° C.



Variables as Functions of pH Value.

FIG. 18.—Volume of powdered gelatin.

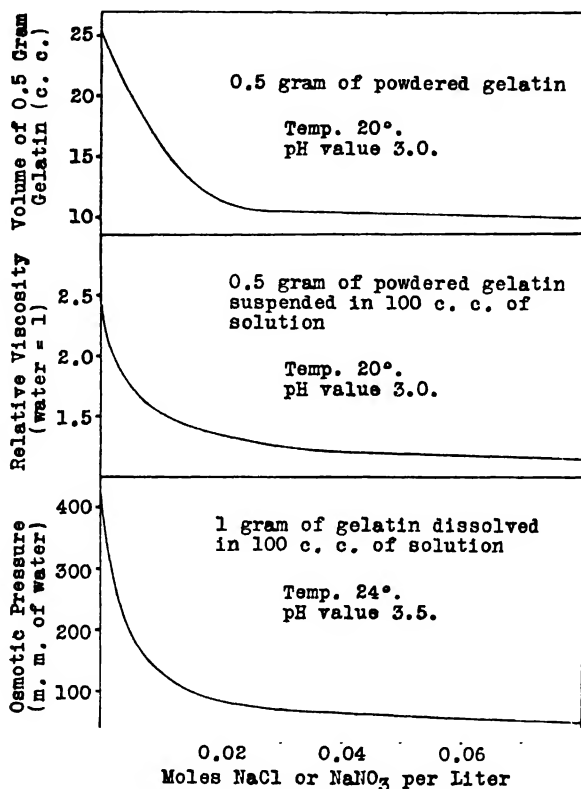
FIG. 19.—Viscosity of gelatin solution.

FIG. 20.—Osmotic pressure of gelatin solution.

solutions of 0.8-per cent gelatin containing different amounts of acid, maintaining this temperature for 1 minute, cooling rapidly to 24°, and immediately determining the viscosity at 24°. The viscosity is plotted against the pH value of the gelatin solution.<sup>29</sup>

In the experiments whose results are shown in Fig. 20, collodion bags, cast in the form of Erlenmeyer flasks having a volume of 50 cubic centimeters, were filled with 1-per cent gelatin solutions con-

taining different amounts of acid. Each bag was closed with a rubber stopper fitted with a glass tube serving as a manometer and put into a beaker containing dilute acid solution of the same-kind as was used in making up the gelatin solution. When osmotic equilibrium was established, the level of solution in the manometer was recorded and



Variables as Functions of Concentration of Added Salt.

FIG. 21.—Volume of powdered gelatin.

FIG. 22.—Viscosity of gelatin suspension.

FIG. 23.—Osmotic pressure of gelatin solution.

plotted against the pH value of the gelatin solution.<sup>33</sup> The measurements were made at 24°.

The explanation of the parallelism between the curves for swelling, viscosity, and osmotic pressure as functions of pH value is that the variation in each case is due to the same fundamental cause, namely, the establishment of a Donnan equilibrium. In the viscosity measurements, the solutions contain aggregate of gelatin molecules capable

of swelling with change of pH value and, since the viscosity must increase with the increasing volume occupied by the gelatin, we should expect the viscosity to rise and fall with the degree of swelling of the gelatin particles.

In the experiments on osmotic pressure, we have an application of the Donnan equilibrium which is considerably simpler than that involved in the swelling of jellies, although of a similar kind.

In the swelling and osmotic pressure experiments, we note that the points of maximum given by sulfuric acid are only half as great as those given by hydrochloric acid, which is in harmony with the theory, since the divalent sulfate ion has no greater diffusion pressure than the monovalent chloride ion and is only half as numerous for equivalent concentrations of gelatin salt.

In Figs. 21, 22 and 23 are given curves showing the depressing effect of increasing concentration of neutral salt upon the volume of powdered gelatin,<sup>34</sup> the viscosity of a suspension of powdered gelatin,<sup>34</sup> and the osmotic pressure of a solution of gelatin.<sup>32</sup> Again we find a parallelism in the results that would be expected from the theory.

### Osmotic Pressure and Membrane Potentials.

A discussion of the mechanism of the osmotic pressures exerted by protein solutions may serve to make the theory of swelling, which is the more important in leather chemistry, a little clearer. The collodion bags used in Loeb's experiments were permeable to water and simple acids, bases, and salts, but not to dissolved proteins. Let us consider a solution of gelatin chloride and hydrochloric acid contained in a collodion bag which is brought into contact with pure water. Hydrochloric acid diffuses out through the membrane until equilibrium is established between the external solution and the gelatin solution inside the bag. The outside solution contains only hydrochloric acid, but the inside solution contains both hydrochloric acid and gelatin chloride. At equilibrium, in the outside solution, let

$$x = [\text{H}^+] = [\text{Cl}']$$

and in the inside solution let

$$\begin{aligned} y &= [\text{H}^+] \\ z &= [\text{gelatin ion}] \\ [\text{Cl}'] &= y + z. \end{aligned}$$

whence

It is apparent from the reasoning given early in this chapter that at equilibrium

$$x^2 = y(y + z)$$

and that

$$2y + z > 2x.$$

The greater concentration of diffusible ions of the inside solution,  $2y + z$ , must give rise to an osmotic pressure proportional to the quantity  $e$  in the expression

$$e = 2y + z - 2x.$$

This assumes that the gelatin exerts no osmotic pressure of its own, which may not be strictly true. A correction would have to be made by adding to  $e$  an amount corresponding to the osmotic pressure of the gelatin. But Loeb<sup>36</sup> has shown that any such correction that may be necessary is less than the probable experimental error of measurement.

When  $x$ ,  $y$ , and  $z$  are determined in the solutions, the osmotic pressure can be calculated. At 24° C. the osmotic pressure, in terms of millimeters pressure of a column of water, equals  $2.5e \times 10^5$ . For casein chloride, Loeb found that the observed osmotic pressure approximated the value  $250000e$  as closely as the determinations could be made.

Because of the unequal distribution of ions between the inside and outside solutions, there must be an electrical difference of potential set up between the two solutions whose measure at 20° C., as in the case of the jellies, is given by the formula

$$\text{P.D.} = 58(\text{pH inside minus pH outside}) \text{ millivolts.}$$

In determining the potential difference between the inside and outside solutions, Loeb used an apparatus similar to that shown in Fig. 16. The collodion bag containing the inside solution was hung in the beaker filled with the external solution. The manometer tube of the collodion bag was replaced by a funnel. The capillary tube of the right hand calomel cell was dipped into the funnel so as to make contact with the inside solution. The potential difference of the system was then measured by means of a Compton electrometer.

Further quantitative proof of the correctness of the theory is furnished by the data in Table XVIII, showing the depressing effect of increasing concentration of neutral salt upon the osmotic pressure and potential difference of a system in which an acid solution of gelatin is separated from a gelatin-free solution by means of a collodion membrane.<sup>31</sup> The osmotic pressure curve is plotted in Fig. 23. When

TABLE XVIII  
GELATIN SOLUTIONS AT 24° C.

Moles NaNO <sub>3</sub> Per Liter	Osmotic Pressure (mm.)	pH Value of		(a) Minus (b)	P.D. (Millivolts)	
		Inside Solution (a)	Outside Solution (b)		Calcu- lated	Ob- served
None.....	435	3.58	3.05	0.53	31.2	31
0.000244 .....	405	3.56	3.08	0.48	28.3	28
0.000488 .....	371	3.51	3.10	0.41	24.0	24
0.000975 .....	335	3.46	3.11	0.35	20.7	22
0.00195 .....	280	3.41	3.14	0.27	16.0	16
0.0039 .....	215	3.36	3.17	0.19	11.2	12
0.0078 .....	134	3.32	3.20	0.12	7.0	7
0.0156 .....	85	3.29	3.22	0.07	4.1	4
0.0312 .....	63	3.25	3.24	0.01	0.6	0

equilibrium was established, the pH values of both inside and outside solutions were determined and the potential differences were determined in the manner described above. The potential differences were also calculated from the pH determinations, the factor 58.8 being used for 24°. The agreement between calculated and observed results is as nearly perfect as could be hoped for.

With increasing concentration of salt, the pH values of the inside and outside solutions approach each other. According to the theory, the distribution of any ion between the two solutions is similarly affected by the addition of salt; i.e., the logarithms of its concentration in the inside and outside solutions, respectively, approach each other, bringing about a lessening of the difference in total concentration of diffusible ions between the two solutions. It is this effect rather than any supposed repression of ionization of the protein salt that is responsible for the reduction in the swelling of jellies and the osmotic pressure, viscosity, and potential difference of protein systems.

#### Changes in Viscosity of Gelatin Solutions with Time.

When hot solutions of gelatin are allowed to cool, their viscosities increase with time until they finally set to rigid jellies. Loeb attributes this to the formation of aggregates of gelatin molecules, the viscosity increasing with the average size of the gelatin particles. The curves in Figs. 24 and 25 show that this increase in viscosity with time is materially influenced both by the pH value and temperature of the gelatin solution.<sup>35</sup> The effect of pH value was determined by rapidly heating 2-per cent gelatin solutions containing different amounts of sulfuric acid to 45° C., cooling rapidly to 20°, and then maintaining this temperature while viscosity measurements were made at intervals



of 5 or 10 minutes. An increasing concentration of acid tends to prevent the formation of aggregates; the viscosity increases most rapidly at the isoelectric point.

The effect of temperature was determined by rapidly heating 2-per cent gelatin chloride solutions having a pH value of 2.7 to 45° C., cooling rapidly to the temperature at which the viscosity measurements were to be made, and maintaining this temperature while determinations were made at intervals of 5 or 10 minutes. The remarkable point

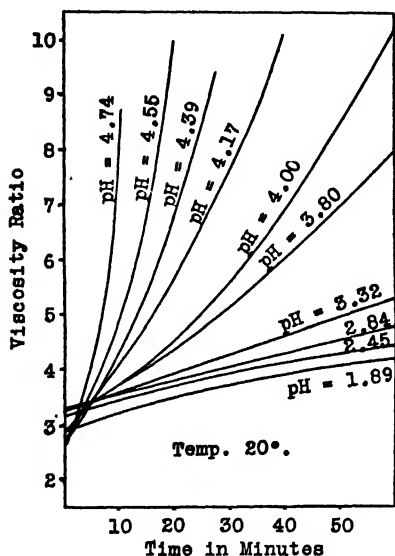


FIG. 24.—Increase in viscosity with time of 2-per cent solutions of gelatin sulfate of different pH values.

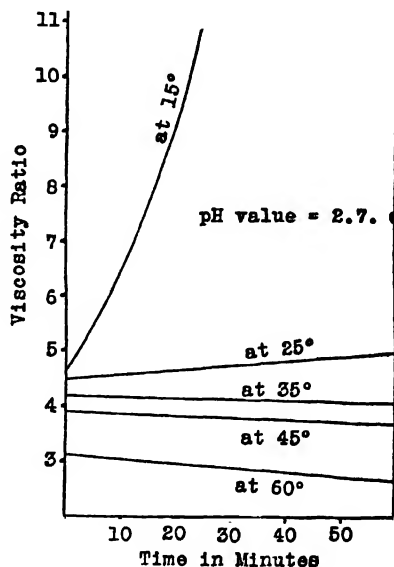


FIG. 25.—Change in viscosity with time of 2-per cent solutions of gelatin chloride at different temperatures.

to be observed is that the viscosity increases with time at temperatures below 35° C., but decreases with time at higher temperatures.

Bogue<sup>3</sup> measured the viscosities of gelatin solutions at different temperatures by means of a Macmichael torsional viscosimeter. At each temperature he made measurements for a number of different speeds of rotation of the cup. A set of these is shown in Fig. 26. The continuous lines cover the range of actual observation and the dotted portions represent the curves extrapolated to zero speed of rotation. For all temperatures above 34° C. the extrapolated curves pass through the origin, indicating truly viscous flow. But for lower

temperatures, the curves do not pass through the origin; they indicate a finite deflection for an infinitesimal speed of rotation, showing that here we have an example of plastic flow. The gelatin solutions at lower temperature actually possess a measurable degree of rigidity. This is further evidence in support of Smith's view that at temperatures above 35° gelatin in solution exists in a form having no power of gelation.

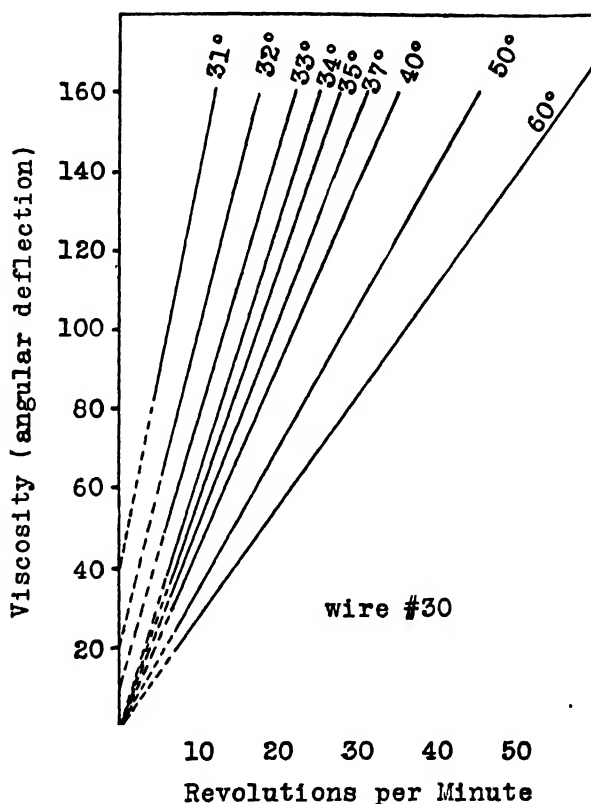


FIG. 26.—Viscosity-plasticity curves for a 20-per cent gelatin solution.

As the temperature is lowered, some of this sol form changes into a gel form which has the power of gelation. As the temperature is lowered, the proportion of gel form to sol form increases until at 15° and lower temperatures all of the gelatin exists in the gel form. The structure of the aggregates of molecules of the gel form is such as to impart to the solution the rigidity observed by Bogue.

When an acid solution of gelatin contained in a collodion bag at 20° C. is brought into equilibrium with a pure aqueous solution of

the acid, the solution actually is separated into 3 phases. The gelatin solution within the bag has a hydrogen-ion concentration less than that of the external solution but greater than that of the solution absorbed by the aggregates of gelatin molecules suspended in the gelatin solution. Loeb<sup>31</sup> has shown that, with increasing proportion of aggregates to dissolved gelatin, the variation of pH value produces an increasing effect upon viscosity, but a decreasing effect upon osmotic pressure measurements, as would be expected.

### Swelling in Pure Water.

The theory given above for the swelling of protein in acid and alkaline solutions has been criticized because it does not explain the swelling of rubber in toluene and other types of swelling. The theory given is not concerned with the swelling of a pure jelly in a pure solvent, but only with the effect of electrolytes on the swelling of protein jellies. Gelatin jellies swell to a relatively small extent in contact with water at the isoelectric point and this swelling is probably the result of the attraction of the molecules of the gelatin for water molecules. An attraction of rubber molecules for toluene molecules is probably the cause of the swelling of rubber in toluene. It is important to differentiate between this type of swelling and the extra swelling resulting from the formation of ionizable protein salts.

### Colloidal Dispersions.

When one material is dispersed in another, the dispersed phase and dispersion medium usually assume electrical charges of opposite sign, due to a shifting of electrons of the outer spheres of the atoms at the interface. In colloidal dispersions in water, or other ionizing media, this shifting of electrons between the dispersed phase and dispersion medium becomes essentially an ionization effect, the group of atoms in the water which receive or give up electrons becoming, in fact, dissolved ions balanced by the corresponding electrical charges on the particles of the dispersed phase. Thus a particle may acquire its electrical charge by ionizing itself, by adsorbing a substance that will ionize, or by adsorbing ions from the solution.

As a rule, the stability of sols increases with the value of the electrical charge on the particles. Two reasons have been given for this effect. The first is that the greater the electrical charge the greater will be the repulsive force between the like-charged particles, tending to prevent their coalescing. The second is that an increasing charge

results in an increasing attraction for water and hence an increasing tendency for the material to become more highly dispersed in the water.

In studying ferric oxide hydrosol, Thomas and Frieden<sup>65</sup> came to the conclusion that the stabilizing effect of adsorbed ferric chloride was due to its forming a solution-link between the otherwise insoluble ferric oxide and water. They found that 1 mole of ferric chloride is required to keep about 21 moles of ferric oxide in stable dispersion regardless of the concentration of the sol. If the stability were due to repulsion of like-charged particles, the limiting ratio of ferric chloride to ferric oxide required to maintain stability should increase with increasing concentration of the particles, since the mutual attractive forces vary inversely as some power of the average distance between the particles. The stability of the ferric oxide sol is thus due to the solution forces of the adsorbed ferric chloride rather than to the repulsive forces of the like charges.

According to this solution-link theory, ferric oxide sol should be soluble in any liquid in which ferric chloride dissolves. Thomas and Frieden found that dilution of the hydrosol with an unlimited amount of alcohol had no effect upon its appearance, nor did the addition of ether to this alcisol precipitate it, provided a large excess was not added.

A particle of this sol may be looked upon as a complex micell made up of aggregates of ionized  $(\text{Fe}_2\text{O}_3)_{21}\text{FeCl}_3(\text{H}_2\text{O})_x$ . The chloride ions pass into true solution in the water, leaving an otherwise insoluble cation behind. The chloride ions have powerful attractions for both the water and for the complex cations. The effect is the same as though the micell had acquired a powerful attraction for water and a corresponding tendency to pass into solution.

This view was developed further by Thomas and Johnson<sup>66</sup> to explain the mutual precipitation of certain hydrosols. The precipitation of one hydrosol by another is usually considered to be an electrical phenomenon in which the positively charged particles of one hydrosol neutralize the negatively charged particles of the other, but the discovery by Freundlich and Nathansohn<sup>14</sup> showing that arsenious sulfide hydrosol and Oden's sulfur hydrosol precipitate each other, although both are negatively charged, is not amenable to explanation by this hypothesis. Lottermoser<sup>41</sup> suggested a chemical mechanism for mutual precipitation of colloids which was based upon a few experiments with silver iodide hydrosol peptized by silver nitrate (or by silver ion) and silver iodide sol peptized by potassium iodide (or by iodide ion). He found the sharpest and most complete mutual

precipitation in those mixtures where the amounts of peptizing silver nitrate and potassium iodide were in, or very near, chemical equivalence.

Thomas and Johnson<sup>66</sup> investigated the coprecipitation of a number of hydrosols and found that the precipitation ratios depended upon the peptizing agents rather than upon the electrical charges. At the point of maximum precipitation, there is chemical equivalence between the peptizing agents of ferric oxide hydrosol peptized by ferric chloride and silicic acid sols peptized by sodium silicate, provided the ratio of peptizing agent to the dispersed phase falls within a certain range, outside of which the precipitation is erratic. Ferric oxide-silicic acid sol precipitations showing chemical equivalence between the peptizing agents at maximum precipitation exhibit little variance in precipitation ratios with dilution, while those showing a divergence from chemical equivalence approach chemical equivalence with dilution. Mutual precipitation of ferric oxide and silicic acid sols is due to the removal of peptizing agents by chemical reactions between them.

### Nature of Surface Potentials.

Up to very recently the view was generally held that an electrically charged particle is surrounded by a so-called Helmholtz double layer, consisting, in the case of a positively charged particle of a layer of positive electricity on the surface of the particle and an equivalent layer of negative electricity forming a thin layer in the liquid immediately surrounding it. This simple picture has been subjected to a vast amount of discussion since the appearance of the classical papers of Quincke,<sup>57</sup> Helmholtz,<sup>17</sup> and Lamb.<sup>24</sup> More recently the view has been advanced that the liquid film containing the balancing charges, carried by ions, contains also ions from the solution at large not balancing any charges on the particles, thus modifying the Helmholtz theory.

In 1916, the author<sup>69</sup> pointed out, in considering the field about an electrically charged particle, that a Donnan equilibrium must exist between the film of solution wetting the particle and the main bulk of solution. As an example, we may consider a gold sol. As has been shown by Beans and Eastlack,<sup>1</sup> when gold is dispersed in water, the presence of chloride, bromide, iodide, or hydroxide ion in concentrations ranging from 0.00005 to 0.005 normal has a marked stabilizing effect on the sol produced and the particles are negatively charged. Apparently these ions are adsorbed by the gold atoms at the surface

or possibly form addition compounds with them. Fluoride, nitrate, sulfate, and chlorate ions decrease the stability of gold sols, which is significant in view of the fact that they do not form stable compounds with gold.

In Fig. 27 let A and B represent two gold particles stabilized by potassium chloride. In combining with the gold, the chloride ions have imparted their negative charges to the particles. But the potassium ions are still left in solution, although their field of motion is restricted to the thin film of solution wetting the particles because they must continue to balance the negative charges on the particles. The volume of the film of aqueous solution enveloping a particle will be measured by the surface area of the particle and the average distance that the potassium ions are able to travel from the surface.

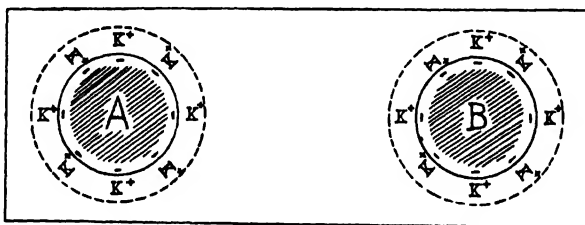


FIG. 27.—Particles of stable gold sol showing enveloping films of aqueous solution.

Let us now consider the case where an amount of potassium chloride is present in the sol too small to cause precipitation. The enveloping film will contain potassium ions balancing the charges on the particles as well as ionized potassium chloride. The surrounding solution will have potassium and chloride ions only in equal numbers. In the surrounding solution let

$$x = [K^+] = [Cl']$$

in the enveloping film let

$$y = [Cl']$$

and  $z = [K^+]$  balanced by charges on the particles,

whence  $y + z$  represents the total concentration of potassium ion.

As was shown in the discussion of Donnan's theory, the product  $[K^+] \times [Cl']$  must have the same value both in the enveloping film and in the surrounding solution at equilibrium. Hence

$$x^2 = y(y + z).$$

The surface layer of solution will have a greater concentration of ions than the surrounding solution by the amount  $2y + z - 2x$ . This un-

equal distribution of ions will give rise to a difference of potential between the enveloping film and the surrounding solution whose measure is

$$E = \frac{RT}{F} \log_e \frac{x}{y} = \frac{RT}{F} \log_e \frac{2x}{-z + \sqrt{4x^2 + z^2}}$$

But now, if we increase  $x$  without limit while  $z$  remains constant,  $E$  must decrease, approaching zero as a limit, since

$$\lim_{x = \infty} E = \frac{RT}{F} \log_e \frac{2x}{\sqrt{4x^2}} = 0.$$

It is thus evident that the difference of potential between the enveloping film and the surrounding solution will be a maximum when there is no free potassium chloride present and will decrease, approaching zero, as the concentration of potassium chloride is increased without limit.

It is important to note here that  $z$  is a measure of the absolute value of the electrical charge on the particles and that it may remain constant while the potential difference rises or falls with corresponding variations in  $x$ .

The particles shown in Fig. 27 are prevented from coalescing because for each there is a sufficiently high potential difference of the same sign between the surrounding solution and each enveloping film. When enough potassium chloride has been added to lower the potential difference below a certain critical value, the particles move toward each other and the enveloping films of two or more particles blend into one, as shown in Fig. 28. It is at this point that the actual charges themselves come into play and probably determine the nature of the precipitate.

It is apparently the difference of potential represented by  $E$  and not the absolute value of the charge that determines the stability of sols of hydrophobic colloids because the surface film completely envelops the particles and endows them with its own properties. Loeb<sup>87</sup> has shown that precipitation of hydrophobic colloids occurs whenever the value of the potential difference falls below about 14 millivolts. The greater the potential difference the greater will be the attraction of the particles for water and the tendency for them to remain dispersed. With dispersions of substances like gelatin and other hydrophilic colloids, which have naturally a great attraction for water, the potential difference may be reduced to an extremely low value, even to zero for some substances, without causing precipitation.

When a mass of gelatin jelly is in equilibrium with a dilute solution of hydrochloric acid, the solution absorbed by the jelly bears a relation to both the insoluble gelatin network on the one hand and the surrounding acid solution on the other, analogous to the relation which the film of solution enveloping a charged particle bears to the surface of the particle and to the main bulk of solution. In the gelatin systems, from simple analyses and the principles of the Donnan equilibrium, it is easy to calculate the potential difference between the jelly phase and the external solution. Loeb's work, described earlier in the chapter, shows remarkable agreement between calculated and observed values. But, since no way was known to isolate and analyze the thin film of solution enveloping a colloidal particle, the same kind of test could not be applied to Wilson's theory of colloidal dispersions.

However, Loeb<sup>37</sup> succeeded in making an indirect test. He rea-

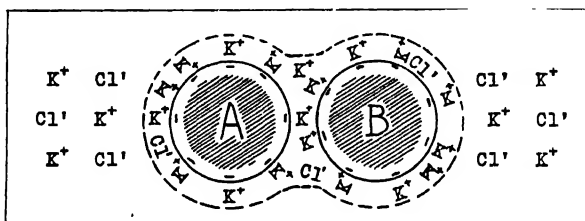


FIG. 28—Coagulation of gold sol initiated by reduction of potential difference between enveloping films and the surrounding solution, by the addition of potassium chloride.

soned, assuming the correctness of Wilson's view, that the depressing effects of salts on the potential difference at the surface of colloidal particles should depend only upon the valency of the ions of the salt and not upon their chemical nature. To test this possibility, the depressing effect of salts with monovalent and bivalent cations on the cataphoretic potential difference of particles of collodion, mastic, and graphite was compared and it was found that the valency rule actually holds. With increasing concentration of salt, the potential difference dropped steadily from initial values in the neighborhood of 55 millivolts, but for equal concentrations it was practically the same whether sodium, potassium, lithium, or rubidium chloride was used at a constant pH value of 5.8. With salts having bivalent cations, the drop was much greater, but for equivalent concentrations, it was the same whether strontium, magnesium, calcium, cobalt, barium, or manganese chloride was used. The theory was thus given some indirect experimental support.



Oakley,<sup>51</sup> too, has since pointed out that a rational explanation of the behavior of a number of colloidal dispersions upon the addition of acids, bases, and salts is afforded by application of the principles of the Donnan equilibrium and the theory of ionization at the surface of colloidal particles as the origin of the electrical charge.

### Theory of Salting-Out.

The theory of stability of colloidal dispersions given above is applicable to the so-called *salting-out* of solutions or dispersions of colloidal materials. It furnishes a needed addition to McBain's<sup>43</sup> theory of soap solutions, in which it would be well to look upon the micelle as an aggregate of monovalent ions rather than as a complex polyvalent ion. It seems likely too that repression of ionization of the soap is not necessary to the salting-out effect. Loeb's work, taken in conjunction with investigations in the author's laboratories, indicates that the lowering of the potential difference of protein systems is not brought about by repression of ionization of the protein salts, as has often been supposed, but rather by the mechanism of the Donnan equilibrium just described. In gelatin systems in which the potential difference has been lowered to a very small value, we find no repression of ionization of gelatin chloride measurable by means of calomel electrodes. Moreover, there is no need to postulate such repression in order to account quantitatively for the observed results.

### Adsorption.

Ever since Gibbs showed that the concentration of the solute must be greater at the surface than in the bulk of solution where the solute lowers the surface tension of the solution, there has been a tendency to look upon this proof as an explanation of the fact that substances of great specific surface reduce the concentration of solute in many different kinds of solution with which they are brought into contact. The error in this tendency lies in the fact that Gibbs' work applies only to the lowering of the surface tension by a substance actually in solution. Since, in many cases, it has not been found possible to determine the actual concentration of solute in the layer of solution immediately in contact with the surface of the material causing a decrease in concentration of solute in the bulk of solution, any conclusions as to the causes of such decrease have been open to question. In the case of gelatin, however, it has been found possible to measure

concentrations in the absorbed solution and this has thrown considerable light on the phenomenon known as adsorption.

Adsorption is a term now widely used to indicate the removal of solute from solution by a material in contact with the solution. An empirical formula was proposed by Freundlich<sup>18</sup> which agrees approximately with some observed results over limited ranges, provided the two constants required in the formula can be selected to suit the findings. The formula may be represented as follows:

$$w = ax^b,$$

where  $w$  is the amount of solute removed from solution by unit quantity of the adsorbing material,  $x$  is the final concentration of solute, and  $a$  and  $b$  are constants selected to suit the occasion. Freundlich mentions that  $b$  may vary from 0.1 to 0.5, but  $a$  very much more.

The very nature of the equation makes it capable of fitting a great variety of data, especially since the constants may be selected as desired, but it doesn't explain anything. Referring back to Table XIV, we find that the total quantity of chloride in the gelatin jelly at equilibrium, represented by  $V(y + z)$ , can be represented as a function of the hydrogen-ion concentration by the use of Freundlich's formula. Letting  $V(y + z) = 7.33x^{0.42}$ , we can plot a curve for the total quantity of hydrochloric acid, combined and uncombined, which has been absorbed by the jelly that agrees fairly closely with both the calculated and observed results given in Table XIV, although not quite so well as do the calculated and observed results with each other. Plotting  $\log V(y + z)$  of the above equation against  $\log x$ , we get a straight line, but the observed results never give a perfectly straight line, but vary in the same directions as do the calculated results of Table XIV.

The curve for the concentration of gelatin chloride shown in Fig. 9 also can be represented approximately by Freundlich's formula by letting  $z = 0.10x^{0.8}$ . The formula is a convenient means of representing a reaction approximately over a limited range, which it is able to do merely because many variables give curves that are nearly parabolic in shape.

Adsorption of ionogens by protein jellies is a manifestation of chemical combination complicated by separation of the system into phases. In the adsorption of chloride ions by a gold surface, the nature of the forces involved is not well understood, but in this, as well as in all other difficultly definable cases of adsorption, it is useful to consider the analogy to the protein-electrolyte equilibrium. In the case of adsorption by dispersions of hydrophobic colloids in water, we

must deal with two phases of the solution analogous to those of gelatin systems, the film of solution enveloping the particles corresponding to the solution absorbed by the gelatin jelly.

For more elaborate treatments of certain phases of modern theories of the physical chemistry of the proteins, the reader is referred to articles by Cohn<sup>5</sup> and Hitchcock<sup>28</sup> and to the books of Loeb<sup>38</sup> and Bogue.<sup>4</sup>



## Chapter 6.

### Microörganisms and Enzymes. .

Tanneries abound with microörganisms. They have been called the tanners' invisible friends and foes. Guarding the skins against damage resulting from their activity is one of the most difficult tasks the tanner is called upon to meet. Only a very inadequate start has been made in the study of these interesting forms of plant life and of their behavior in the tannery, but through intensive research we hope some day to control their activities in the tannery just as accurately as it is now possible to control some of the chemical processes.

The four great subdivisions of plant life are the *Spermatophyta* or seed plants, the *Bryophyta* or moss plants, the *Pteridophyta* or fern plants, and the *Thallophyta*, which include bacteria, yeasts, and molds, the common microörganisms of the tannery.

The *Thallophyta* are simple plants which are never differentiated into roots, stems, and leaves. Those which contain chlorophyll are the *Schizophyta* or blue-green algæ; those unicellular plants which multiply by cell fission only and contain no chlorophyll are known as the *Schizomycetes* or bacteria. Those unicellular or multicellular plants which multiply by means other than simple cell fission and contain no chlorophyll are the *Fungi*, which include yeasts and molds, or mildews.

#### Bacteria.

A *bacterium* is a plant consisting of a single cell containing no chlorophyll and reproducing only by cell fission. Bacterial cells are usually one of three shapes, spheres, straight rods, or bent rods. A spherical cell is a *coccus*; a straight rod a *bacillus*; and a bent rod a *spirillum*. Bacteria are so small that it is customary to take as the unit of measurement the micron, which is one-thousandth of one millimeter. The dimensions of common bacteria range from 0.5 to 10 microns. A bacterium of average size might have a volume of one cubic micron, so it would take one trillion bacteria to fill the space of one cubic centimeter. Of all the liquors in the tannery, those most heavily laden with bacteria are the bate liquors and the highest count

ever obtained in the author's laboratories of a bate liquor was slightly more than one billion per cubic centimeter. This tremendous number occupies only about 0.1 per cent of the volume of the liquor and probably weighs not more than one milligram.

Bacteria multiply by a process of cell fission. The cell grows until one dimension has practically doubled, when it separates into two individual cells. Some bacteria produce spores in the course of their growth. The spore is usually much more resistant to destruction than the parent cell and the theory has been advanced that spore formation is not a method of multiplication, but rather a protection against unfavorable environment.

During the multiplication of bacteria, the cells do not always become detached from each other, but often cling together forming characteristic groups which serve to identify them. Such group formation of long chains of rod-shaped forms is shown in Plate 47, which is a photomicrograph of *bacilli* found in a used tannery soak water. The cells simply become elongated and then subdivide, but remain attached forming long chains. When spherical cells divide in this manner, forming long chains, they are called *streptococci*. When they form irregular clusters, they are called *staphylococci* and when they show a tendency to remain united in pairs, they are called *diplococci*. *Spirilla* very rarely occur in groups.

Some bacteria possess organs of locomotion known as flagella. These are extremely thin protoplasmic threads and serve to propel the organism by means of a corkscrew motion. The bacilli shown in Plate 48 are equipped with flagella. For any particular organism the size, number and arrangement are characteristic and serve to identify it.

Buchanan <sup>4</sup> has made an interesting calculation of the rate of growth of bacteria under ideal conditions. Probably most of the more active bacteria can grow to their full size and divide to form two individuals within thirty minutes. If it is assumed that this process continues for two days the number of bacteria growing from one initial cell will be 2 raised to the 96th power and their total weight more than one trillion tons. Of course no such bacterial masses could ever be formed because of the disappearance of available food and the production by the bacteria of substances hindering their own development.

Bacterial activity involves the diffusion of dissolved foods through the cell walls of the bacteria, reactions within the cells, growth and reproduction, and the formation of substances which diffuse out from

the cell into the solution in which the bacteria are suspended. Often these excreted materials are of very great importance. Since the collagen of skin is insoluble, it cannot diffuse into the bacterial cell, but the cell may secrete a proteolytic enzyme which will hydrolyze the collagen, forming soluble products which can diffuse through the bacterial wall and into the cell, where they help to form more of the same enzyme to diffuse out and hydrolyze more collagen. Bacterial cells of different kinds may secrete a large variety of active substances.

### Bacterial Damage to Skins.

Skins must be protected against bacterial action from the moment of flaying until they are fully tanned. Freshly flayed skins quickly pick up bacteria from their surroundings and the soluble proteins contained in the skin furnish ideal food for the proteolytic bacteria, which multiply rapidly and secrete enzymes capable of destroying the collagen fibers of the skin. Methods of protecting skins against such damage until they reach the tannery will be discussed in Chapter 7.

In spite of the precautions ordinarily taken, many skins suffer from bacterial damage. Although the harm occurs before the skins have been tanned, it may not be apparent until after tanning. Damages are most common on skins which have not been properly cured, skins which have been stored in too warm a room, and skins which have been kept too long in warm or dirty water. The conditions which determine the nature of the damage are, however, not well understood. There seems to be a special type of damage peculiar to each given set of conditions. For example, in some cases only the grain surface seems to be affected, in others only the regions close to the blood vessels, and in still others only the glandular region separating the thermostat and reticular layers of the skin. Examples of these have recently been illustrated by Wilson and Daub.<sup>10</sup>

Plates 41 and 42 show vertical sections cut from a chrome-tanned calf skin which appeared to be covered with freckle-like spots after tanning and finishing. The surface of the skin had been attacked by colonies of bacteria distributed all over it. Plate 41 shows an unattacked region between spots and Plate 42 one of the spots. None but the thin fibers on the very surface have been attacked and only to a depth of less than 0.01 millimeter. The spots, however, appear very prominent after finishing and glazing because of the dark luster they assume. Although the damage was done before tanning, it was not noticeable until after the finishing operations.

Plate 43 shows a similar type of damage, but it was confined entirely to the hair follicles of the skin. The tiny fibers lining the follicles had been severed and partially destroyed. In the later operations the cavities formed, became clogged with dirt and excessive amounts of finishing materials, with the result that the leather appeared spotted after finishing, which greatly lessened its market value.

A very troublesome defect in some kinds of leather made from light skins is the appearance of the pattern of the blood-vessel system on the surface, usually referred to as veins. It becomes noticeable after the leather is dried and finished. Plate 45 shows a section of veiny chrome calf leather cut at right angles to the line of direction of a vein. It will be seen that the fibrous region surrounding the vein has been eaten away. When the leather is glazed, it receives less pressure wherever these channels occur. Since the darkening of the skin upon glazing increases with the pressure applied, the result is a light-colored pattern on the surface of the skin corresponding to that of the blood-vessel system below it. This trouble occurs frequently on improperly cured skins which have suffered some putrefaction. It is remarkable, however, that the blood-vessel system may be strongly attacked when there is no noticeable damage to the fibers of the grain surface. This suggests that the blood left in the skins is the source of infection. Plate 46 shows a section of another piece of chrome calf in which the vein runs parallel to the plane of cutting. Here the damage has been accentuated by the glazing operation. Although the losses in money value, due to the veiny condition, are greatest for light upper leathers, they are not inconsiderable even for the heaviest hides.

Plate 44 represents a section of vegetable-tanned calf leather showing the cause of loose grain. When the skin was still untanned, the bacteria had attacked the region of the sudoriferous glands and severed most of the fibers holding the thermostat and reticular layers of the skin together. The fibers of the grain surface are, however, unattacked.

The tanner is familiar with a number of general methods for retarding bacterial action. Skins that are to be kept a long time before tanning are sometimes dried; bacteria do not develop in the absence of water. In curing skins, high concentrations of salt are used, which practically stop bacterial activity. In soaking skins, low temperatures are used which are unfavorable to rapid growth of bacteria. Antiseptics which destroy bacteria or inhibit their activity are sometimes employed. Another method, which promises to become of very



great importance in checking bacterial action on putrescible materials, is the control of pH value of the solutions in contact with the putrescible materials.

### Effect of pH Value.

Merrill and Fleming, in the author's laboratories, studied the effect of pH value on bacterial damage to raw skin. In order to get liquors of definite pH value, they titrated a thirtieth-molar solution of phosphoric acid with sodium hydroxide, following the titration with the hydrogen electrode. The phosphate served as a buffer to prevent wide fluctuations of pH value after the strips of skin were introduced. These were taken from skins either after soaking or after bating and placed in solutions of definite pH value, kept at 25° C. in a thermostat. From time to time the strips of skin were examined. In the case of the soaked skins, bacterial action was measured by the ease of slipping of the hair; in the case of the bated skins by the pitting of the grain.

At pH values less than 3.0, the strips suffered from acid hydrolysis. Between 3.0 and 4.5, the acid caused a loosening of the hair, but there was no pitting and no evidence of any bacterial action. Between 4.5 and 6.5 there was no hair slipping and no pitting noticeable. Between 6.5 and 8.0 both pitting and hair slipping became marked, with a maximum action at about 7.5. The actions were quite marked after two days. At pH values above 8, no pitting took place. The action on the hair follicles, resulting in hair slipping, decreased gradually as the pH value rose from 7.5 to 10.5, above which it increased again, apparently due to the action of the alkali rather than of bacteria.

These experiments would indicate that there is greatest danger of bacterial damage when skins are kept in contact with solutions having pH values between 6.5 and 8.0.

### Making Bacterial Counts.

Interesting information regarding bacterial development in many tannery liquors can be obtained by making counts and the method commonly used in America is that of the American Public Health Association. For actual working details, the Association's book<sup>1</sup> of methods should be consulted. It is sufficient here to discuss the principles of the method.

A sample of the tannery liquor is taken in a previously sterilized bottle and dilutions with sterilized water are made as quickly there-

after as possible. Where the bacteriologist has no idea as to what the count may be, he makes the following dilutions: 1; 10; 100; 1000; 10,000; 100,000; 1,000,000; 10,000,000; and 100,000,000 times. As soon as any dilution is made, the sample is shaken vigorously and then a portion is drawn off to make the next dilution, in proper order. At the same time another portion is plated. This is done by mixing 1 cubic centimeter of the sample or dilution with 10 cubic centimeters of liquefied culture medium at 40° C. in a Petri dish and then allowing the mixture to solidify as rapidly as possible. Where a medium containing gelatin has been used, the plates are kept in an incubator at 20° C. and where agar has been used, the plates are kept in a 37° incubator. Of course, care must be taken that no organisms other than those present in the original sample are introduced, and the details of the method aim to guard against such contamination. The gelatin plates are incubated for 48 hours and the agar plates for 24 hours. Both incubators are kept dark, well ventilated, and practically saturated with moisture.

At the end of the incubation period, the colonies visible on each plate are counted, using an engraver's lens of 2.5 diameters' magnification, with a focal length of 3.5 inches. Plates containing from 30 to 300 colonies are preferred in making the official count. It is assumed that each colony grew from one organism in the original sample.

As an example, we may take the count made on some water used for soaking a pack of salted calf skins. On the agar plates at 37° C., for all dilutions less than 1000 times, the colonies were too numerous to count. On the plate with the dilution of 10,000 times, there were 102 colonies; on the dilution of 100,000 times 11 colonies; and on the dilution of 1,000,000 times only 2 colonies. Since 102 lies between the preferred numbers 30 to 300, it was chosen as official and 102 multiplied by 10,000, the value of the dilution, gives 1,020,000, the number of organisms present in 1 cubic centimeter of the original sample. But the count on the gelatin plates at 20° C. was 12,500,000, indicating that only about 8 per cent of the organisms capable of growing in gelatin at 20° were able to grow in agar at 37°. This may be due to the fact that the temperature of these soak waters never exceeds 15° in practice and that the organisms developing at the greatest rate in the soak water were incapable of development at 37°. Incubating for a longer time had no effect. The average of counts made over a period of a year showed about 1,000,000 at 37° and 12,000,000 at 20°.

A water dosed with 44 parts of chlorine per million before the skins were soaked showed counts after soaking as follows: at 20° 28,600 and at 37° 33,000, indicating that the chlorine had acted more effectively on the organisms growing at the lower temperature.

The difference in count of the same sample made at different temperatures or with different culture media is often so large as to raise the question as to just what is meant by the count. No one claims that an official count of 1000, for example, means that the sample contains only 1000 bacterial cells per cubic centimeter. It does mean that it contains at least 1000, but it may contain very many more. The counts have a comparative value when made under as nearly identical conditions as possible. The chemist who would use an official method in the investigation of a practical process must understand the limitations of the method or he may be led into difficulties. This is illustrated by the work of Wilson and Vollmar<sup>21</sup> on the effect of salt upon the bacterial count of tannery soak waters.

### Effect of Salts.

When bacterial counts were made of water used for soaking salted calfskins, much higher results were obtained by substituting sterilized water from the soaks for distilled or tap water in making the necessary dilutions. At first this was attributed to the creation of an environment more nearly like that in which the bacteria had originally developed, but further investigation showed it to be due to the salt present in the dilution water.

It was found that salts may cause either an increase or decrease in bacterial count, depending upon kind and concentration present in the culture medium, whether added directly or carried in by the sample being tested. Where counts are used in the study of the effect of antiseptics upon bacterial life in water of variable salt concentration, it is obviously of great importance to know the extent to which the salt may vary the count. For this reason, an investigation was made of the effect of the addition of different salts to the culture medium upon the bacterial count of water used for soaking salted calfskins, and a few typical results are here given.

All counts were made by the official method of the American Public Health Association, except for deviations hereinafter noted. The soak waters whose counts are given in this paper contained an average of about 0.03 mole of sodium chloride per liter, but this was reduced to a negligible value by the dilution, before planting, with

sterilized distilled water, necessary to bring the number of colonies on each plate within the required limits. Duplicate series were run in every case with the soak water diluted both 10,000 and 100,000 times before planting.

Bacto \* nutrient agar was used as a base for the culture medium. No attempt was made to differentiate between the combined and uncombined inorganic components of this medium, but the same sample was used in all experiments. The salts, previously dissolved in water,

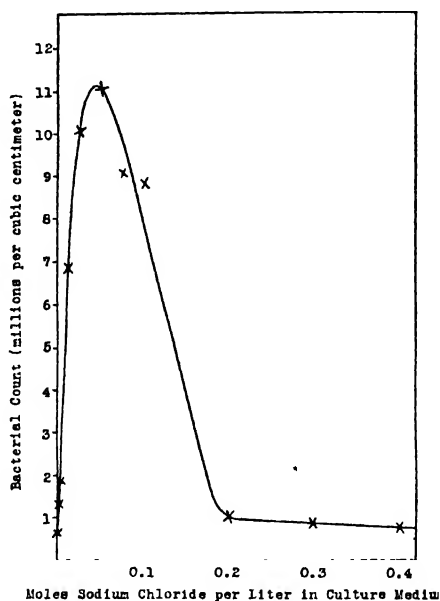


FIG. 29.—Effect of concentration of sodium chloride upon the bacterial count of water used for soaking salted calfskins.

were added directly to the medium before making up to final volume and sterilizing. The importance of controlling the hydrogen-ion concentration was appreciated, and determinations were made in every case after incubation as well as before. In no case did the pH value lie outside of the range 6.90 to 7.10. Microscopic observations were made of many pure cultures obtained from samples of soak waters, but the variety was so great that detailed descriptions would be out of place here.

The effect of increasing the concentration of sodium chloride is shown in Fig. 29. All counts are for the same sample of soak water,

\* Digestive Ferments Co., Detroit.

the only difference being that of salt concentration in the culture medium. Using the official method, with no added salt, the count was 610,000 per cubic centimeter, but with increasing concentration of salt the count rose to a maximum of 11,100,000 at 0.05 mole per liter of added salt, and then fell steadily, becoming practically zero above molar strength. The addition of 0.05 mole of sodium chloride per liter to the medium caused the appearance of eighteen times as many colonies as in the unsalted medium. That this was not due merely to

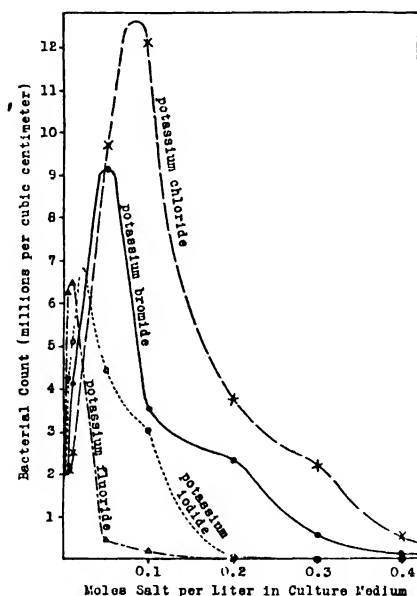


FIG. 30.—Effect of concentration of potassium halides upon the bacterial count of water used for soaking salted calfskins

an accelerated growth was indicated by the fact that incubation for a week, instead of 48 hours, did not alter any of the counts. Care was also exercised to insure against effects of manipulation in planting a large number of aliquots from the same diluted sample of soak water, by making alternate plantings in salted and unsalted media. The effect of repeated pipetting from the same sample was a gradual increase in count in the unsalted medium from 610,000 to 960,000—quite negligible compared with the increase to 11,100,000 due to 0.05-molar sodium chloride. Numerous repetitions showed the effect to be general.

The curves in Fig. 30 furnish a comparison of the effects of the

different halides of potassium upon the bacterial count of another sample of soak water. At concentrations of 0.01 mole per liter, or less, the salts all increase the count, the order of effectiveness being  $KF > KI > KBr > KCl$ . Points of maximum occur in all curves, and at higher concentrations the salts all decrease the count, the order of effectiveness being exactly the same as for increasing count at lower concentrations. Where the initial rise in the curves is steepest,

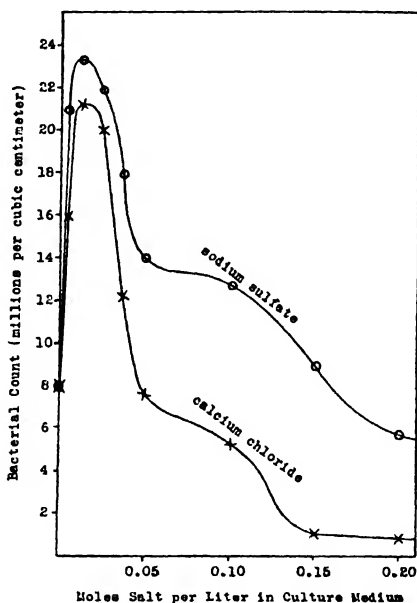


FIG. 31.—Effect of concentration of sodium sulfate or calcium chloride upon the bacterial count of water used for soaking salted calfskins.

the value of the point of maximum is lowest and occurs at the lowest concentration of salt.

A third sample of soak water was used to determine the effects of sodium sulfate and calcium chloride upon the count. The results are shown in Fig. 31. The general shape of all curves is the same and all seem to show curious points of inflection to the right of the point of maximum.

It was at first suggested that the effect of the salt was to produce a condition more or less favorable to the growth of certain types of bacteria, but this appeared improbable when the following facts were brought to light and considered collectively:

1—When the experiments were repeated for short portions of the curves, making successive increments of salt very small, it was found that the curves were practically continuous and that each small increment caused a corresponding increase or decrease in count.

2—Increasing the period of incubation caused no change in the number of colonies on any given plate.

3—A violent shaking of the diluted sample immediately before planting in the medium caused a large increase in count. An example of this follows: A sample shaken 15 seconds gave a count of 280,000 per cubic centimeter; 1 minute, 670,000; 2 minutes, 1,220,000; 3 minutes, 1,790,000; 5 minutes, 2,430,000. The count was increased more than eight times simply by shaking the sample vigorously for 5 minutes before planting.

A more logical explanation is that probably all the bacteria present develop upon incubation, at least in the more dilute salt solutions, but that differences in count are due to differences in the average number of bacteria (originally present in the sample) responsible for one colony on the Petri dish. The bacteria in the sample would thus be pictured as existing in groups or clusters of many individuals. The effect of a small amount of salt would be to increase the degree of dispersion of the bacteria, which would result in an increased number of colonies on the Petri dish, even though the total number of individual bacteria had not been altered by the salt; still larger amounts of salt would cause agglutination and correspondingly lower counts. Violent shaking causes an increase in count by breaking up the clusters, at least temporarily, into smaller groups.

This view likens the behavior of suspensions of bacteria to that of colloidal dispersions of simple materials, such as metallic gold, in the presence of electrolytes. Loeb<sup>11</sup> studied the effect of concentration of different types of ionogens upon the electrical potential difference at the surface of the particles in various kinds of colloidal dispersions. The effect of concentration of sodium chloride upon a colloidal dispersion of gold, for example, was to increase the negative potential difference to a maximum at a concentration of about 0.005 mol of sodium chloride per liter, above which the value of the potential difference fell. Northrop and De Kruif,<sup>14</sup> on the other hand, showed that the electrical potential difference at the surface of bacteria was markedly affected by changing concentration of electrolyte, just as in the case of colloidal dispersions. Loeb found that when the absolute value of the potential difference at the surface of particles of gold, graphite,

collodion, and some other materials, in colloidal dispersion, fell below about 15 millivolts, flocculation occurred, while Northrop and De Kruif found a similar critical potential difference, about 15 millivolts, for the agglutination of certain types of bacteria. Still further confirmation of the view that bacteria behave towards electrolytes much like ordinary colloidal dispersions is furnished by the work of Winslow, Falk, and Caulfield<sup>22</sup> on the electrophoresis of bacteria.

Apparently, in both bacterial suspensions and colloidal dispersions there are opposing forces at work tending to regulate the average size of the particles, whether these be groups of bacteria or aggregates of simple molecules. Cohesive forces act to increase the size, while like electrical charges and attraction of the molecules of the particles for water tend to decrease the average size of the particles. Since it has been shown that the potential difference at the surface of bacteria is altered by change in the kind and concentration of salt, other things remaining constant, one would expect the average number of bacteria per group, and therefore the bacterial count, to be a function of the kind and concentration of salt present in the suspension, and this has been shown to be the case. It seems likely that all counts indicated in the curves are lower than the true values, and it may be questioned whether the full count of individual bacteria in a sample is ever obtained by use of the official method. In making comparative counts, the importance of maintaining constancy of pH value in the culture medium is now generally appreciated, but this work indicates that constancy of composition in other respects is also essential.

### **Examination of Pure Cultures.**

In making bacterial counts, only the relatively huge colonies visible to the naked eye are seen. In order to view the individual organisms, special technic is required. An ordinary plate used in counting may contain a great variety of organisms, making complete identification of all organisms present in a tannery liquor a very long and tedious task. The end of a thin platinum wire is touched lightly upon the surface of a colony in the Petri dish and then is stirred in a drop of water on a clean glass slide so as to distribute the bacteria evenly over the surface of the slide. The smear is allowed to dry in the air and then passed quickly through a flame, smear side up, two or three times to fix the smear. The glass slide is then dipped into a solution of the appropriate stain, washed thoroughly in running water, dried, and examined directly with the oil immersion lens. The immersion

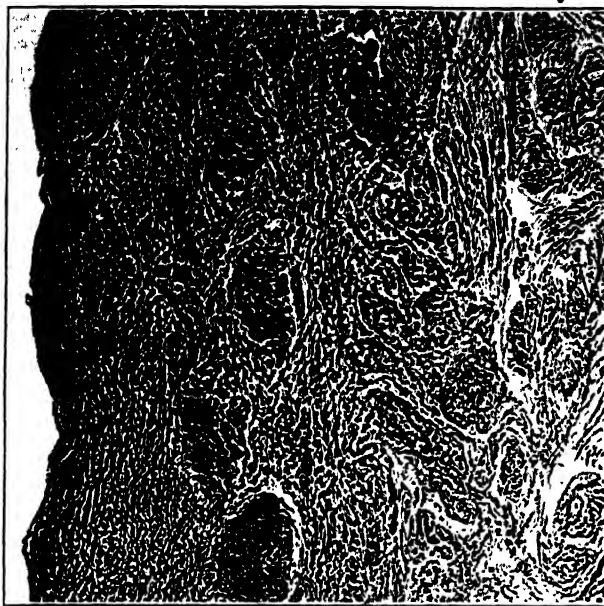




**Plate 42.—Vertical Section of Calf Leather.**

(Showing attacked portion of grain surface.)

Location: butt. Eyepiece: 5X.  
 Thickness of section: 20  $\mu$  Objective: 8-mm.  
 Stain: none. Wratten filter: K3-yellow.  
 Tannage: chrome. Magnification: 250 diameters.



**Plate 41.—Vertical Section of Calf Leather.**

(Showing unattacked portion of grain surface.)

Location: butt. Eyepiece: 5X.  
 Thickness of section: 20  $\mu$  Objective: 8-mm.  
 Stain: none. Wratten filter: K3-yellow.  
 Tannage: chrome. Magnification: 250 diameters.

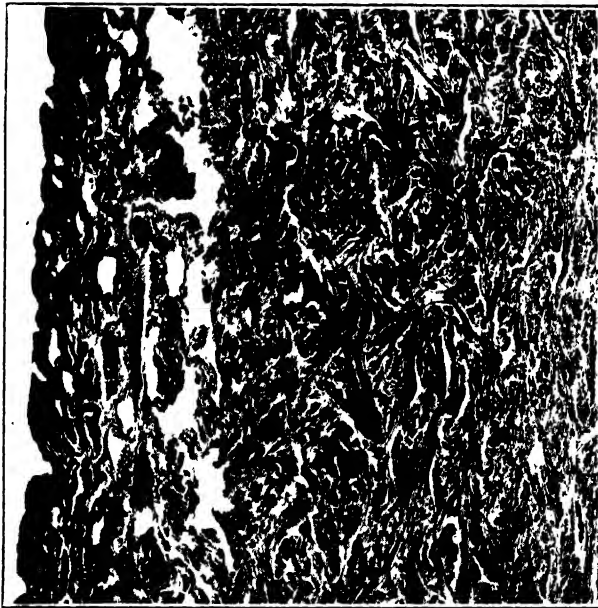


Plate 44.—Vertical Section of Calf Leather.

(Showing cause of loose grain.)

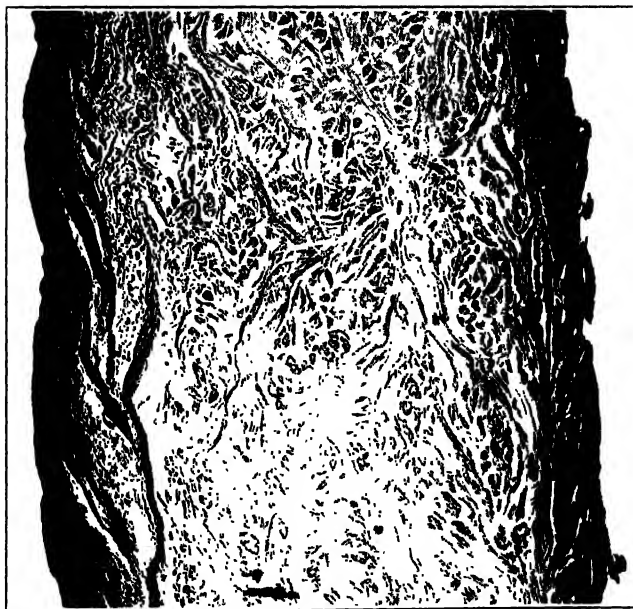
Location: backbone  
 Thickness of section 30  $\mu$   
 Eye-piece: none  
 Objective 16-mm  
 Stain: none.  
 Tannage: vegetable  
 Watten filter: H-blue green  
 Magnification 50 diameters



Plate 43.—Vertical Section of Calf Leather.

(Showing damaged hair follicle)

Location: butt  
 Thickness of section 30  $\mu$   
 Eye-piece: 5X.  
 Objective 16-mm  
 Stain: none.  
 Tannage chrome  
 Watten filter: K3-yellow.  
 Magnification 120 diameters.



**Plate 46.—Vertical Section of Calf Leather.**

(Cut parallel to loose vein)

Location: butt  
 Thickness of section 40  $\mu$   
 Stain: none  
 Tannage: chrome.  
 Excipice: none  
 Objective: 16-mm  
 Wratten filter: K3-yellow  
 Magnification: 50 diameters.



**Plate 45.—Vertical Section of Calf Leather.**

(Cut at right angles to loose vein.)

Location: belly.  
 Thickness of section 40  $\mu$   
 Stain: none.  
 Tannage: chrome.  
 Excipice: none.  
 Objective: 16-mm.  
 Wratten filter: K3-yellow.  
 Magnification: 50 diameters.



**Plate 47.—Long Chains of Rod-shaped Bacilli from Tannery Soak Water.**

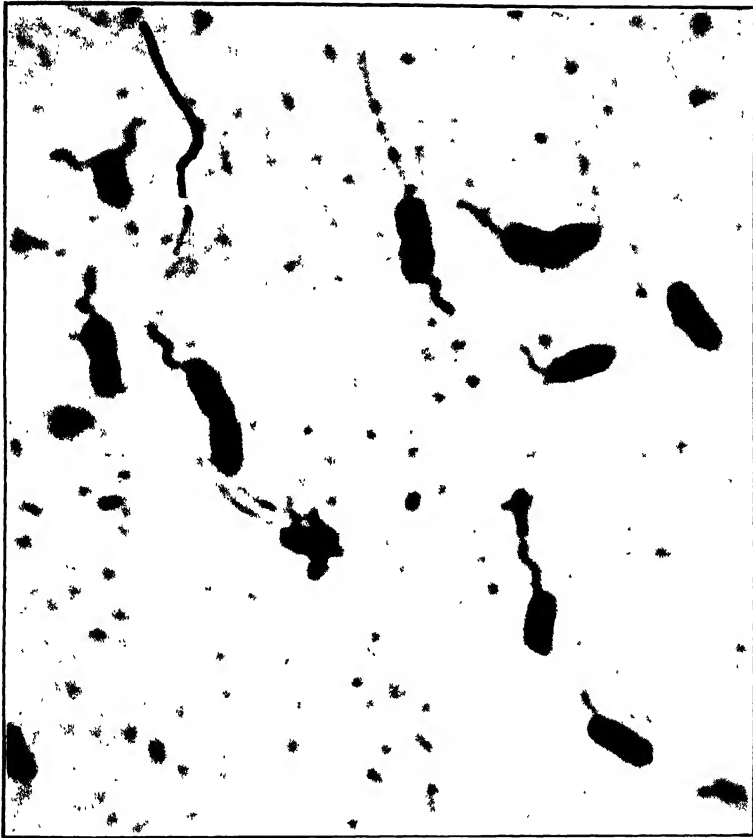
Stain: Loeffler's methylene blue.

Eye-piece: 7.5X.

Wratten filter: E2-orange

Objective: 1.9-mm oil.

Magnification: 1600 diameters



**Plate 48.—Rod-shaped Bacilli with Flagella from Tannery Soak Water.**

Stain: Williams' method.

Eye-piece: 25X.

Wratten filter, E2-orange

Objective: 1.9-mm. oil.

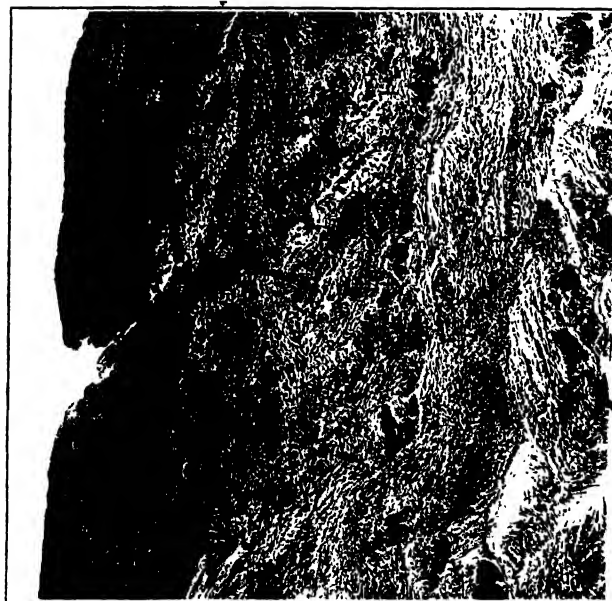
Magnification: 2700 diameters.



**Plate 50.—Vertical Section of Calf Leather.**

(Showing black mold growing among fibers.)

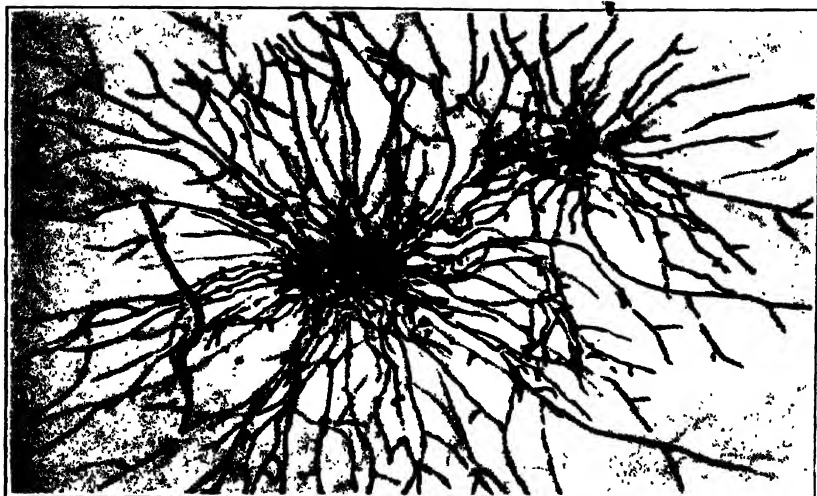
Location: butt. Eyepiece: 5X  
 Thickness of section: 30  $\mu$ . Objective: 8-mm.  
 Stain: none. Wiatren filter: K3-yellow  
 Tannage: vegetable Magnification: 250 diameters



**Plate 49.—Vertical Section of Calf Leather.**

(Showing black spot caused by mold.)

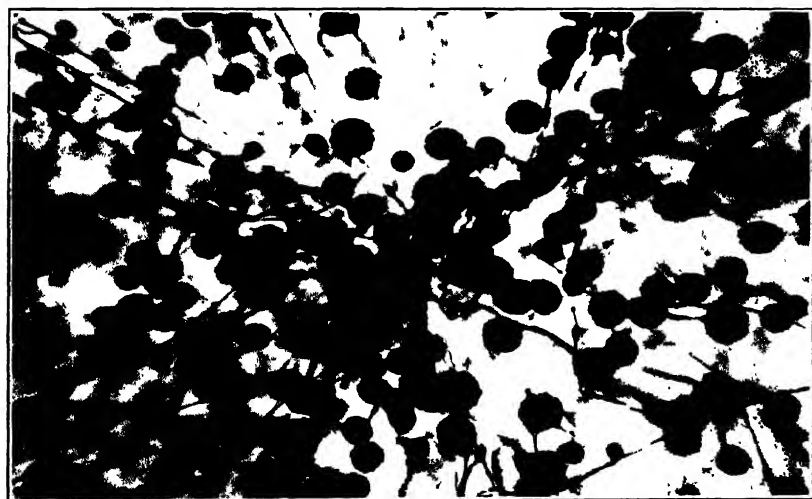
Location: butt. Eyepiece: 5X  
 Thickness of section: 30  $\mu$ . Objective: 16-mm.  
 Stain: none. Wiatren filter: K3-yellow  
 Tannage: vegetable Magnification: 90 diameters



**Plate 51.—*Aspergillus Niger* from Spotted Leather.**

Medium: potato dextrose agar  
 Temperature: 37° C  
 Period of growth: 16 hours.  
 Stain: none

Eye-piece: 12.5X  
 Objective: 32-mm.  
 Wratten filter: K3-yellow  
 Magnification: 37 diameters.



**Plate 52.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 51 except for period of growth.)

Medium: potato dextrose agar  
 Temperature: 37° C  
 Period of growth: 40 hours  
 Stain: none

Eye-piece: 12.5X  
 Objective: 32-mm.  
 Wratten filter: K3-yellow  
 Magnification: 37 diameters.



**Plate 53.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 51 except for magnification.)

Medium: potato dextrose agar.

Temperature: 37° C.

Period of growth, 16 hours

Stain: none.

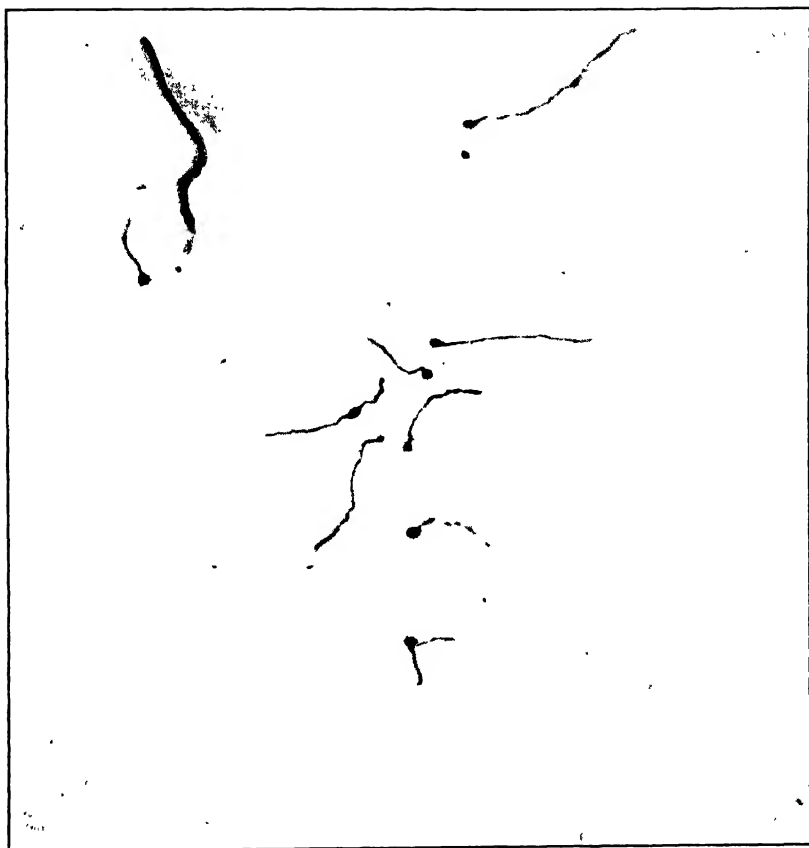
Eye-piece: 12.5X

Objective: 16-mm

Wratten filter, K3-yellow

Magnification 280 diameters.





**Plate 54.—*Aspergillus Niger* from Spotted Leather.**

Medium: potato dextrose agar  
 Temperature 25° C  
 Period of growth 24 hours.  
 Stain: none

Eye-piece. 12.5X  
 Objective 32-mm  
 Wratten filter K3-yellow  
 Magnification 50 diameters.



**Plate 55.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 54 except for period of growth)

Medium: potato dextrose agar.

Temperature: 25° C

Period of growth: 26 hours

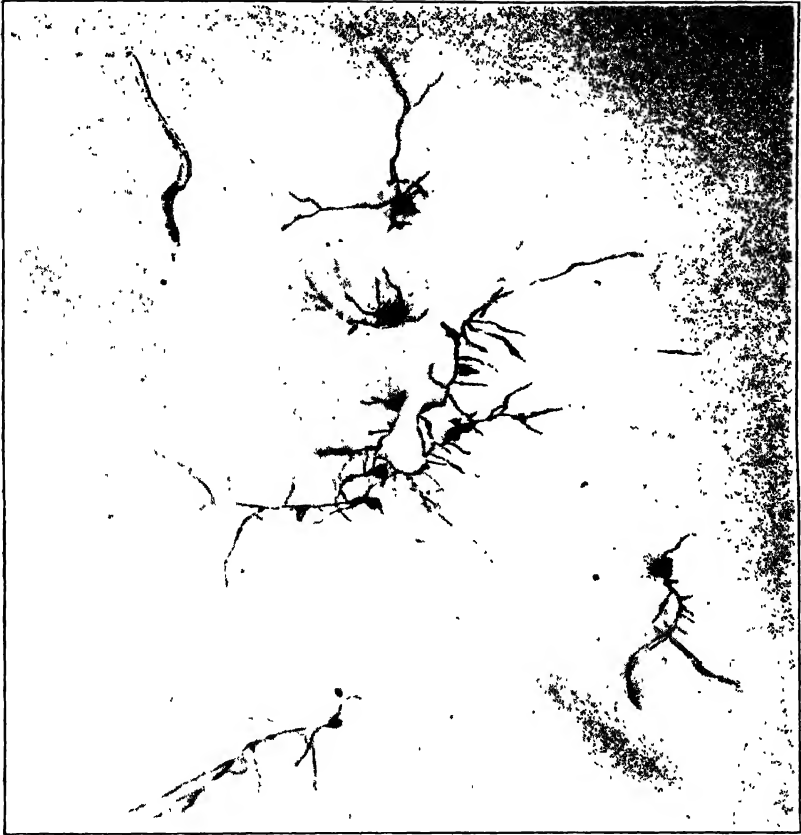
Stain: none

Eye-piece: 12.5X.

Objective: 32-mm

Wratten filter: K3-yellow

Magnification: 50 diameters



**Plate 56.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 54 except for period of growth.)

Medium: potato dextrose agar

Temperature 25° C

Period of growth: 28 hours

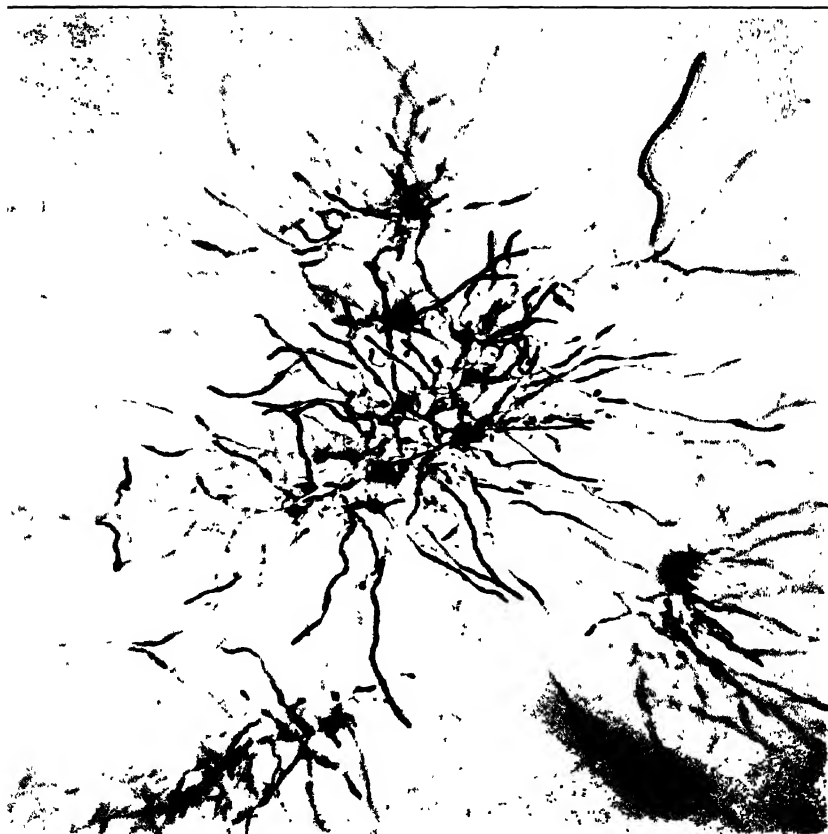
Stain: none.

Eye-piece, 12.5X.

Objective 32-m.

Wratten filter: K3-yellow

Magnification 50 diameters

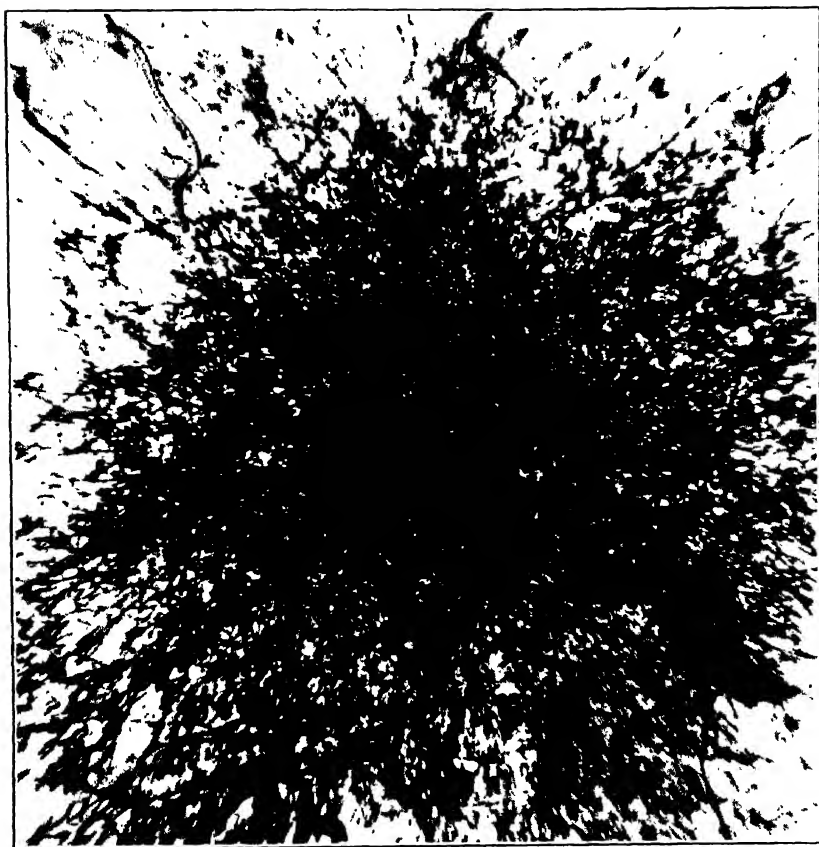


**Plate 57.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 54 except for period of growth )

Medium: potato dextrose agar  
Temperature: 25° C.  
Period of growth: 325 hours.  
Stain: none

Eyepiece: 12.5X  
Objective: 32-mm  
Wratten filter: K3-yellow.  
Magnification: 50 diameters.

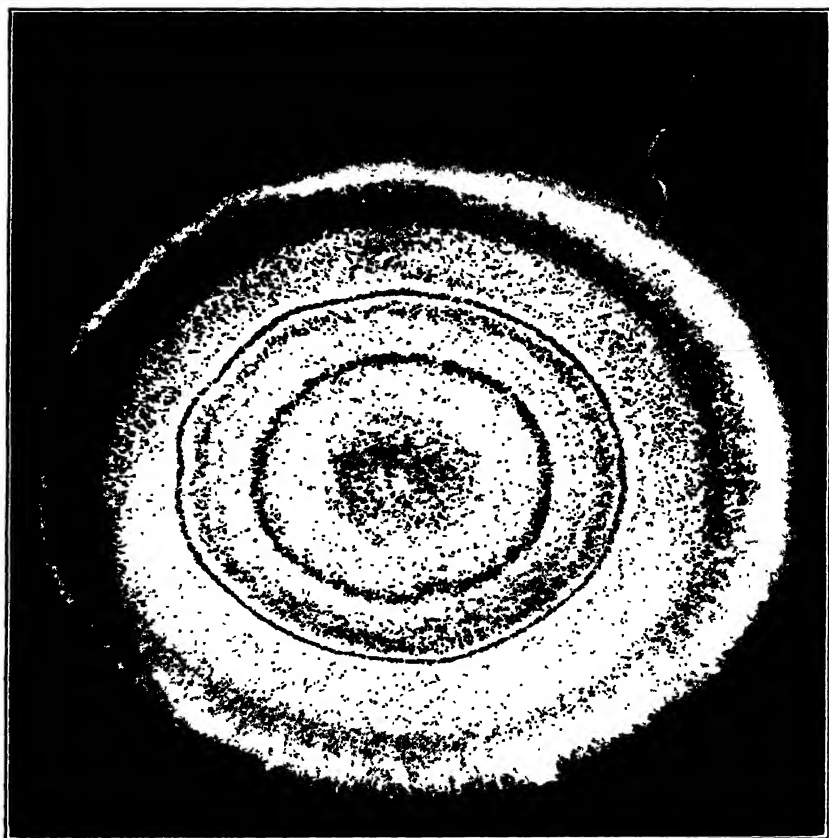


**Plate 58.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 54 except for period of growth )

Medium: potato dextrose agar  
Temperature: 25° C  
Period of growth: 48 hours  
Stain: none.

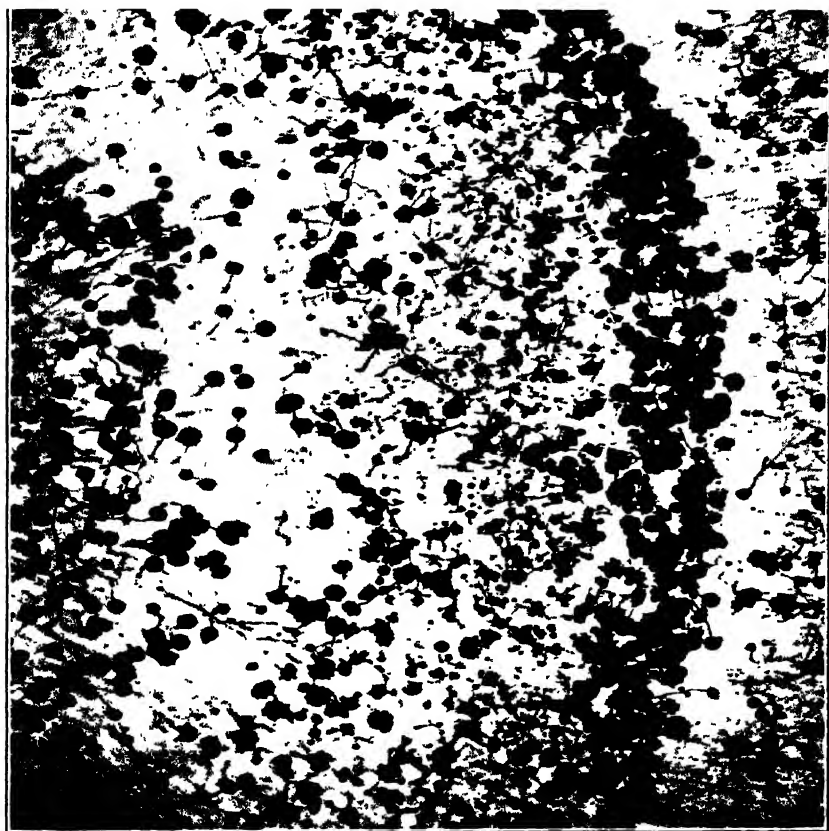
Eye-piece 12.5X.  
Objective: 32-mm.  
Wratten filter: K3-yellow.  
Magnification: 50 diameters.



**Plate 59.—*Aspergillus Niger* from Spotted Leather.**

Medium potato dextrose agar  
 Temperature 37° C  
 Period of growth 6 days  
 Stain none

Eye-piece: none  
 Objective Gradlex lens  
 Wiatfen filter none.  
 Magnification 18 diameters



**Plate 60.—*Aspergillus Niger* from Spotted Leather.**

(Same as Plate 59 except for magnification.)

Medium: potato dextrose agar  
 Temperature: 37° C  
 Period of growth: 6 days  
 Stain: none.

Eye-piece: none  
 Objective: 32-mm.  
 Wratten filter: none  
 Magnification: 14 diameters



Plate 61.—Heads of *Aspergillus*  
*Fumigatus*.



Plate 62.—Heads of *Aspergillus*  
*Terreus*.

(Magnified 260 diameters.)



Plate 63.—Spores of *Macrosporium* or *Alternaria*.



oil may be removed with xylol and the slide retained as a permanent mount.

There are many methods of staining in use for identifying the different kinds of bacteria. Loeffler's methylene blue, used in staining the bacteria shown in Plate 47, and Williams' method, used to stain the flagella pictured in Plate 48, are described in detail in Mallory and Wright's book.<sup>12</sup> For working details of bacteriological technic, reference must be made to the books devoted to bacteriology.

After staining and mounting, the bacteria can be seen with a good microscope equipped with an oil-immersion objective. Plates 47 and 48 indicate the kind of picture to expect.

### Yeasts.

Yeasts are also organisms consisting of a single cell each, but the cells are usually larger than those of bacteria and the yeast cell usually reproduces by a process of budding. The true yeasts are known as *Saccharomycetes* and their cells are spherical or ellipsoidal in shape, varying in diameter from 2 to 12 microns. They are all capable of producing spores under suitable conditions, which vary with the species. The yeasts of the genus *Schizosaccharomyces* do not multiply by budding, but by a process of fission.

One of the properties of yeasts most widely known and used is their power to convert sugars into alcohol and carbon dioxide by the process known as fermentation. Yeasts act directly only upon monosaccharide sugars and on those containing an even number of carbon atoms, such as hexoses, but yeasts are capable of producing enzymes which readily convert polysaccharides into monosaccharides. Some yeasts produce maltase, which hydrolyzes maltose to two molecules of dextrose; others produce sucrase, which hydrolyzes cane sugar to dextrose and levulose; and still others produce the enzyme lactase, which hydrolyzes milk sugar into dextrose and galactose.

Species of yeasts are differentiated by their ability to ferment particular sugars, the effect of air upon their growth, the development of spores, and the rate at which they can bring about fermentation under fixed conditions.

In the tan yard, yeasts bring about fermentation of the sugars introduced in the tanning extracts, but the alcohol formed gradually becomes oxidized to acid, which assists in the tanning process. This oxidation seems to result from the activity of certain acid-forming bacteria.

### Molds.

Foodstuffs, cloth, leather, and most of the organic materials of our everyday life become covered with a growth of mold when left exposed to damp air for several days. The ease with which mold spores are blown about makes their appearance in the atmosphere of common occurrence. In the tan yard, liquors which have not been agitated for two or three days are usually covered with a soft, velvety carpet of molds of various colors. Leather kept in a damp condition is almost sure to become covered with mold in the course of a few days. Mold is a source of considerable annoyance to tanners because it produces spots on leather which cannot be removed by any practical means known at present without damaging the leather itself. Since preventive measures must be used to guard the leather against mold damage, it is highly desirable that those who handle leather much should know something about the life and habits of molds as well as how to keep them from spotting the leather.

The danger of bacterial damage is greatest before the skin has been tanned, but mold spots may appear on shoes or leather in any other form at any time after it has been dampened. These spots are not uncommon and appear as varied in size, shape and distribution as freckles on the human face. When leather has been dampened and contaminated with spores from the atmosphere or from the watery solution used, it is likely to develop spots after damping again at any later date. After the first damping, the molds begin to grow, but they may not have time to develop into visible spots before the leather is dried again. With each subsequent wetting, an opportunity is afforded for further growth until the spots become pronounced.

Wilson and Daub<sup>19</sup> made a detailed study of spots produced on leather by molds. Plate 49 shows a section of calf leather cut through the center of a black, freckle-like spot, one of thousands which covered the skin. The skin itself is not attacked, but the spot is due to the presence of a black mold. Plate 50 shows a portion of the same spot at higher magnification so that individual cells may be seen. The spots resisted every kind of chemical treatment tried which did not harm the leather. However, in a test piece the spots disappeared when the leather was kept under ideal conditions for mold growth for a month; it was then completely covered with a heavy layer of mold, but when this was brushed off, the spots were gone. The procedure was interesting, but hardly practical.

Molds present a more complex structure than bacteria or yeasts,

being multicellular and having a more complicated method of reproduction. The molds consist of two kinds of cells, assimilative and reproductive. In their growth, they form a cobweb-like mass of branching threads from the surface of which tiny fertile threads project into the air bearing the part of the plant from which the spores develop. The main mass of branching threads is called the *mycelium* and an individual thread a *hypha*. The species of mold is generally told by the nature of the spore-bearing or fertile hypha. In most molds the mycelium is *septate*; that is, the hyphæ are divided by cross walls, called *septa*.

The Buchanans<sup>3</sup> list five general families of common molds: (1) *Mucoraceae*, whose spores are frequently borne in a spore case, called a *sporangium*, and whose mycelia are often nonseptate. (2) *Mucedinaceae*, whose spores, called *conidia*, are never borne in a sporangium, whose mycelium is septate, whose fertile hyphæ or *conidiophores* are not united into definite bodies, and whose hyphæ are not dark or smoky. (3) *Dematiaceae*, like Family 2 except for the fact that either or both the hyphæ and conidia appear dark. (4) *Stilbaceae*, whose conidiophores are united into stalks or bundles. (5) *Tuberculariaceae*, whose conidiophores are united into a definite layer or stratum.

Since it is possible here to give only a brief description of molds and their behavior on leather, it seems best to follow Wilson and Daub<sup>20</sup> in their study of *Aspergillus niger*, isolated from spotted leather.

*Aspergillus niger* belongs to the family *Mucedinaceae* and is differentiated from other members of the family by having the following characteristics: Its conidia are one-celled and are borne in chains and its conidiophores are sharply differentiated from the mycelium and inflated at the apex. The conidiophores are unbranched and usually relatively long. At the swollen apex there are numerous short stalks, called *sterigmata*, usually set close together, giving the conidiophore the appearance of a war club with spikes. These sterigmata are relatively short, practically always branched, and from their tips the chains of spores are differentiated.

### Preparation of Cultures for Study.

Wilson and Daub obtained their cultures for study from leather showing black spots. The surface of each strip of leather was first passed back and forth quickly through a flame. The strip was then

suspended from a cork in a bottle over water protected against continuous contamination from the outside air. In the course of about a week each strip was covered with a miniature forest of black mold which had grown out from the interior of the leather. Innumerable tiny threads projected out from the leather, each supporting a ball of black spores.

By means of a platinum loop, some of these spores were transferred to tubes of Bacto potato-dextrose agar and incubated at 37° C. Fresh cultures were made from time to time by inoculating new tubes with spores from the older ones and the purity of each culture was assured by making platings and comparing the individual colonies.

For study under the microscope, glass slides were fitted with culture chambers, into each of which was put about 5 drops of the agar medium. A few spores were transferred from a culture tube to 10 cubic centimeters of Bacto beef broth liquor and shaken well and then a tiny quantity of this liquor was transferred to the center of the culture chamber by means of a needle. The culture chamber was sealed from the air by means of a removable cover glass. All of the customary precautions were taken to prevent contamination of the cultures being studied. At intervals during their growth, the cultures in the glass culture chambers were photographed for permanent record.

For details of the best modern methods for studying molds, the reader is referred to the recent book of Thom and Church.<sup>17</sup>

Plate 51 is a photomicrograph of the mold in one of these chambers which was kept in an incubator at 37° C. for 16 hours after inoculating. Plate 52 shows the same mold after 40 hours at 37°. Plate 53 is the same as Plate 51 except for the higher magnification. Here the septa in the mycelium are plainly visible.

### Nature of Growth.

The development of spores is illustrated in Plates 51 and 52. From the branching mycelium shown in Plate 51, fertile hyphae have developed upward and fruited with the formation of spherical spore masses shown in Plate 52, each such mass having a diameter of about 0.15 millimeter and each individual spore a diameter of about 0.004 millimeter.

Plates 54 to 58, inclusive, illustrate the growth of the mold from individual conidia or spores. Culture slides were inoculated and watched under the microscope. The need for keeping the slide under the microscope at all times prevented incubation at the optimum

temperature for the growth of this mold, which is from 33° C. to 37° C. Since the temperature averaged about 25° C., the cultures did not grow nearly so rapidly as that shown in Plates 51 to 53.

Plate 54 shows nine spores 24 hours after inoculation. They have already sent out little branches, the beginning of the formation of their mycelium. The further development during the next few hours is pictured in Plates 55 to 58. Actually one can project the mold onto the ground glass of the camera and watch it grow. At the end of 48 hours, the picture has changed to that shown in Plate 58; on the surface of leather, this would have been a black spot about two millimeters in diameter.

When the mold is allowed to spread out freely on a culture plate, the fruiting stalks, or conidiophores, arrange themselves in rhythmic, concentric circles. The center of a Petri dish containing potato-dextrose agar was inoculated as in the case of the culture chamber containing the spores shown in Plate 54. The dish was kept in the incubator at 37° C. for six days, when the colony had grown to a diameter of about five centimeters. It is pictured at low magnification in Plate 59 and a portion of it at higher magnification in Plate 60. The mycelium does not show because the focusing was done on the spore heads.

### Industrial Importance.

*Aspergillus niger* is important commercially in the fermentation of sugars to gallic and citric acids. Under anaërobic conditions, it will convert sugars into alcohol. It secretes, under favorable conditions, a great variety of enzymes, of which Thom and Church<sup>17</sup> list the following: lipase, amylase, inulase, raffinase, gentianase, zymase, melezitase, invertase, maltase, trehalase, cellobiase, emulsin, urease, protease, nuclease, rennet, and the enzyme tannase, which has been blamed for losses of tannin in the tan yard. This formidable list of enzymes would indicate that it is not safe to let *Aspergillus niger* run wild in the tannery.

Thom and Church examined tan liquor from a fermenting vat and found the predominating mold to be *Aspergillus niger*, with minor admixtures of a species of *Penicillium* (*Citromyces*) and yeast. Van Tieghem's identification and description of *Aspergillus niger* resulted from his study of fermenting tan liquors and form the beginning of our real knowledge of the biochemic importance of the *Aspergilli*. Knudson<sup>8</sup> found a progressive increase in tannase with the addition of tannin in Czapek's solution with 10 per cent sugar, with *Aspergillus*

*niger* as the fermenting agent. Maximum production of tannase occurred when 2 per cent tannic acid had replaced all of the sugar in the formula.

*Aspergillus niger* thrives in acid solution and grows particularly well on chrome leather. The author has observed this mold growing vigorously on chrome calf leather, which was neither fatliquored nor colored, when immersed in any strength of pure sulfuric acid solution up to and including 1.5-normal. It did not grow in 2-normal acid or stronger. Its growth was quickly stopped by immersion in 0.1-normal sodium hydroxide solution, indicating that an alkaline medium is not favorable to its development.

### Prevention of Mold Spots.

Since the mold does not grow appreciably on dry leather, the best preventive of spots is to keep the leather from getting and remaining damp. Where the wetting of the leather cannot be avoided, it should be dried again as quickly as practicable. Many of the spotted and mottled effects which appear on leather during prolonged rainy periods are due to molds. Molds are present in the air almost everywhere ready to inoculate leather the minute it becomes wet. For this reason, keeping the leather dry is the most effective preventive of mold damage.

However, mold damage can be aggravated by carelessness and uncleanliness. Where a liquid is to be applied to leather, the danger from damage by molds is greatly increased if this liquid is already heavily contaminated by molds. Where there is danger of such contamination, disinfectants should be used.

Of the common disinfectants studied by Wilson and Daub, the most effective against *Aspergillus niger* was an aqueous solution of chlorine. In a contaminated mixture, 1 part by weight of chlorine to 50,000 parts of water killed the mycelium and fruiting portions of the mold in ten minutes. Para-chlor-meta-cresol has about one-fourth of the disinfecting power of free chlorine, per unit weight. Mercuric chloride was found to be less effective than free chlorine, requiring from ten to twenty times as much to do the same work. Formaldehyde has been suggested as a disinfectant for molds, but it was found necessary to use 1500 times as much as of chlorine to be effective. A precaution always to be taken where leather is stored is to keep the rooms clean, disinfected, and reasonably dry.

### Other Molds.

Thousands of species of molds have been described, but the great majority of those found in the tannery belong to the two groups: *Aspergilli* and *Penicillia*. Wilson and Daub isolated 12 species of mold from a single strip of leather, which were identified as follows by Drs. Charles Thom and Margaret B. Church, of the Bureau of Chemistry, United States Department of Agriculture: Five were species of *Aspergillus*; five were species of *Penicillium*; one was a species of *Macrosporium* (or *Alternaria*); and one a species of *Brachysporium*. The species of *Aspergillus* were *niger*, *flavus*, *fumigatus*, *terreus*, and *nidulans*. Two of the species of *Penicillium* belonged to the group known as *divaricatum* (Thom: *Paccilomyces varioti* Bainier). Both *Penicillium* and *Aspergillus* belong to the family *Mucedinaceae*.

The *Macrosporium* and *Brachysporium* belong to the family *Dematiaceae*. In this same family is the species *Dendryphium*, whose conidia grow in chains. In a study never carried far enough to justify publication, Wilson and Daub isolated a species of *Dendryphium* from spotted leather and made a few preliminary tests which indicated that this species of *Dendryphium* was even more to be feared than *Aspergillus niger* in the spotting of leather.

Plate 61 shows the heads of *Aspergillus fumigatus*, Plate 62 the heads of *Aspergillus terreus*, and Plate 63 the spores from a species of *Macrosporium*. These are published through the courtesy of Drs. Charles Thom and Margaret B. Church.

It is easy to distinguish *Penicillium* from *Aspergillus* by the manner in which the spore-bearing structures are borne. In *Penicillium*, the conidiophores branch in whorls, giving rise to a terminal cluster of parallel threads, of which each ultimate branchlet is to be regarded as a sterigma. From each of these a chain of conidia is developed. The branches and conidia together resemble a broom or brush. The name *Penicillium* is Latin for little brush. Several hundred species of both *Aspergillus* and *Penicillium* are known.

In these few pages, it would be futile to attempt to do more than to indicate to the reader, in a general way, what molds are and how they act and to refer him to books which treat the subject systematically. An excellent work on *Penicillium* was published by Thom<sup>16</sup> in 1910. Very recently a splendid book on the *Aspergilli* has appeared by Thom and Church.<sup>17</sup> These books will be found very useful to the leather chemist who is called upon to study molds in the tannery, as these two great groups of molds are to be found everywhere. Much

help can also be found in the key to families and genera of common molds in the book by the Buchanans,<sup>3</sup> where 141 species are described. A further valuable aid is the American Type Culture Collection, which sells pure type cultures at a nominal fee. This collection of molds is supervised by Drs. Charles Thom and Margaret B. Church of the Bureau of Chemistry. Type cultures of bacteria, yeasts, and molds are obtainable from the collection through Dr. George H. Weaver, the John McCormick Institute for Infectious Diseases, 637 South Wood St., Chicago, Ill.

How to deal with molds, even after they have been identified, is not always an easy problem. A mold that is harmful in one respect may be helpful in another. In an unpublished work, Wilson and Daub tried to get rid of the molds causing spots on leather by destroying them in the tan liquors. They had no difficulty in producing mold-free liquors, but invariably the liquors which were not covered by a layer of mold rapidly darkened through oxidation and the leather tanned in it was of an undesirably dark color. The molds apparently were preventing too rapid an oxidation of the liquors. Of course, the molds may also modify the fermentation of tan liquors and the production of desirable acids.

### **Enzymes.**

Among the products secreted by microorganisms is the class of substances known as enzymes. Bacteria cannot act directly on insoluble protein material like collagen. They absorb the soluble foods through their cell walls. Complex reactions go on within the cell and among the products formed and secreted are enzymes. The enzyme then may act upon collagen, breaking it down into soluble products, which may diffuse through the cell walls of the bacteria, furnishing food. Enzymes are also secreted in abundance by the organs of higher animals. The pancreas of pigs and other animals is the source of the enzyme trypsin used by the tanner in bating.

An enzyme is a chemical substance formed by the life process of a living organism which is capable of increasing or decreasing the speed of some chemical reaction without itself being appreciably used up during the reaction; in other words, it is a catalyst. A few milligrams of enzyme may bring about chemical reactions involving many pounds of material. Although produced by living matter, enzymes are not living organisms, but they become inert, as catalysts, when heated to temperatures above 60° C. while wet. Their chemical com-



position and structure are unknown because it has not yet been found possible to isolate the pure substances. What we know of enzymes has been learned from a study of the action of materials containing enzymes upon the chemical reactions which they catalyze.

Among the most important reactions catalyzed by enzymes are those taking place during the digestion of food in the animal body. The saliva, the gastric juice, and the intestinal juices contain enzymes which break down the various foods into simpler substances which can be assimilated by the body. The substance upon which an enzyme acts is called its *substrate* and the accepted system of nomenclature is to name the enzyme after the substrate upon which it acts. Thus fat-splitting enzymes are known as *lipases*, protein-hydrolyzing enzymes as *proteases*, and starch-hydrolyzing enzymes as *amylases*. Some enzymes are so extremely selective as to act upon only one substrate, even to the exclusion of one of a pair of optical isomers. Others, less discriminating, act upon all members of a whole class of compounds, although at different rates.

The enzymes of greatest importance to the tanner are the proteases. Two enzymes of this class, pepsin and trypsin, are distinguished by the different ranges of pH value in which they are active rather than by the substrates upon which they act. Pepsin, which is found in the stomach, acts only in an acid medium, pH values 1 to 4, while trypsin, found in the pancreas, acts in neutral or slightly alkaline solutions, pH values 5 to 10. There is a definite pH value of greatest activity for the action of each enzyme upon each substrate. The optimum value for trypsin is usually found at about  $\text{pH} = 8$ , which is of great importance to the tanner because this is approximately the pH value of soak waters, bate liquors, and the solutions present in the skin during curing.

The animal pancreas, dried, ground and defatted is known as pancreatin. It contains amylases and lipases, as well as trypsin. Any one class of enzymes can be concentrated or separated very roughly from the others by a series of precipitations with organic liquids, or other means, for details of which the reader is referred to such treatises as those of Euler<sup>8</sup> and Oppenheimer<sup>15</sup> or the monographs of Falk<sup>6</sup> and Bayliss.<sup>2</sup> When the proteases of the pancreas are thus concentrated, they are marketed as trypsin in order to distinguish them from the less highly purified pancreatin. But even the most active tryptins of the market are far from pure and any distinction between trypsin and pancreatin is of value only in indicating relative purity.

Since trypsin acts on many proteins, such as gelatin, casein, elastin,

etc., many authors regard it as consisting of as many different enzyme substances, called gelatase, caseinase, elastase, etc., respectively. It is possible, however, that trypsin may be composed of a number of different enzymes, each of which acts on a number of different proteins, although not at the same rate. How widely different the activity of different samples of pancreatin or trypsin on different proteins may be is beautifully illustrated by the values recorded in Table XXIX of Chapter 10.

The analysis of an enzyme preparation consists largely in measuring its activity upon different substrates. In Chapter 10 methods are described for making such measurements and evaluating the enzyme.

Regarding the mechanism of enzyme action, the prevailing opinion seems to be that the enzyme first combines chemically with one of the reactants, forming a compound more reactive than the original reactant and thus hastening the reaction. But, in the course of the reaction, the enzyme is again liberated so as to combine with more of the original reactant. The laws governing the kinetics of such reactions appear to be the same as those governing ordinary, simple chemical reactions.

The activity of an enzyme may be influenced by or even totally dependent upon the presence of other material; for example, a small amount of ammonium chloride increases the activity of trypsin on elastin, while a large amount decreases it. Enzymes in solution gradually lose their activity, the more quickly the higher the temperature. The speed of a reaction catalyzed by an enzyme increases with rise of temperature, just as do other chemical reactions, but this is offset by the increasing degree of inactivation of the enzyme with rising temperature and the result is that the apparent rate of reaction is a maximum at some definite temperature. For most enzyme actions, the greatest activity is observed at about 40° C. The speed of a reaction catalyzed by an enzyme increases with increasing concentration of the enzyme, although in direct proportion only over a limited range.

The properties of enzymes are discussed further in Chapters 9 and 10 in connection with unhairing and bating.



## Chapter 7.

### Preservation and Disinfection of Skin.

Practically every country in the world supplies hides and skins for leather manufacture. The skins from large, fully grown animals are usually called *hides*, those from half grown animals of the larger variety *kips*, while those from small or very young animals, or those intended for furs, are called *skins*. For example, as the calf grows into a cow, its skin remains a skin until it reaches a weight of about 15 pounds in the wet state, when it becomes a kip, while it becomes a hide at about 30 pounds. These figures are necessarily arbitrary, but serve to indicate the general scheme of classifying skins according to size. A bull hide may weigh more than 100 pounds. A sheep skin always remains a skin because it never assumes great size. The skin of the full grown East Indian buffalo is called a kip because it is smaller than the ordinary cow hide. For convenience, the term skin is used in its general sense throughout this book to include hides and kips, except when referring to specific cases.

Skins are sold under the names packer, city butcher, or country, depending upon where the flaying was done. Packing houses usually engage experts for the various tasks of flaying, grading, and curing and this makes the packer skins better on an average than the others. An interesting account of the methods of selection and grading hides and skins in commerce will be found in a paper by Stubbe and Levi.<sup>87</sup>

The fact that animals are generally raised and slaughtered for food rather than for purposes of leather manufacture makes the tanner's chief raw material a by-product of the packing industry. For this reason a decreasing consumption of leather has but little influence upon the continued supply of skins, although it does tend to lower their market value. On the other hand, a brisk demand for leather generally does not in itself stimulate the raising and slaughtering of cattle, but rather has the effect of increasing the vigilance against damage to the existing supply of skins by putrefaction, careless handling, or the ravages of insects. Raw skins are highly putrescible and, since a considerable period of time usually elapses between the slaughter and

the first tannery operation, it is necessary to subject them to some method of preservation as soon as possible after flaying.

How long the various parts of the skin continue to live and function after the animal has been flayed remains to be determined. We do know, however, that the skin undergoes changes of one sort or another practically from the moment of flaying. McLaughlin<sup>12</sup> noted that the rate of swelling of hide in saturated lime water decreases during the first two or three hours following the flaying of a freshly killed animal. A strip of hide put into lime water containing undissolved lime in excess 30 minutes after flaying swelled about 30 per cent more in 120 hours than a corresponding strip put into the lime water 210 minutes after the flaying.

This is, of course, not surprising in view of the fact that many changes are known to occur in skin, after the death of the animal, all of which would tend to retard the swelling in lime water. The coagulation of the blood, during which fibrinogen is converted into fibrin, would tend to retard the penetration of lime into the skin and the partial drying of some of the tissues would act in a similar manner. Decomposition of some of the protein constituents would yield simpler bodies capable of forming salts of calcium, which would serve to repress the swelling of the proteins by calcium hydroxide. It is possible also that some of the proteins capable of swelling are gradually broken down into simpler bodies not having the power to swell.

Where the preservation of a skin has been done carefully and intelligently, these changes appear not to have any detrimental effect upon the leather produced. The author has tested this by comparing the tannage of skins properly preserved and kept for months before tanning with the tannage of skins put into process within an hour of the death of the animals; no advantage in working the fresh stock could be detected by chemical, physical, or microscopical examinations of the final leathers. But where there is carelessness in handling, the skins may suffer irreparable damage before the soaking operation has been completed.

In bacterial action of a certain type, one of the first effects to be noticed is the loosening of the hair, a condition known to the trade as hair-slippiness. Either the bacteria, or the enzymes which they secrete, act upon the soft epithelial cells of the Malpighian layer of the epidermis, liquefying them and thus effecting a separation of the whole of the epidermis and hair from the rest of the skin. This action alone is not harmful, but the bacteria develop rapidly and soon begin to attack the fibers in the grain surface and the skin is permanently

injured. This effect shows itself in the finished leather in the form of dull spots, or what is known as pitted grain. In some cases the bacteria attack the heavier collagen fibers without injuring the fibers of the grain surface. When the bacteria attack the proteins of the thermostat layer, they weaken the connection between the fibers of the grain surface and those of the reticular layer; in the finished leather the grain surface then tends to peel off and its looseness of connection with the main body of the skin gives it the appearance known as pipy grain. A number of bacterial damages to leather are pictured in the preceding chapter.

### Salting or Curing.

The commonest method of preserving skins, where they do not have to be transported very long distances and where salt is reasonably cheap and plentiful, is salting or curing, as it is called in the trade. The salt checks bacterial action, which would otherwise cause irreparable damage. In the work of Wilson and Vollmar, described in Chapter 6, it was found that bacteria from one tannery soak water would not develop in a 6-per cent salt solution, even under the ideal conditions involved in making bacterial counts. When skins are properly cured, they are usually free from bacterial damage, but it must not be supposed that concentrated salt solutions stop the activity of all kinds of bacteria. Harrison and Kennedy<sup>9</sup> investigated the red discoloration of cured codfish and found it to be due to the growth of a red bacterium (*Pseudomonas salinaria*) found in the so-called solar or sea salts and capable of growing in saturated salt solutions.

In order to cure skins so that practically no bacterial damage will occur upon long storage, a great many precautions must be taken, some of which are only imperfectly understood. Since the appearance of the first edition of this book, McLaughlin and his coworkers have done much to improve our knowledge of the curing of skins, developing both the theoretical and practical aspects.

When animals are flayed in the packing houses, it is customary to leave the skins lying in a hide cellar until the body heat has been dissipated. Then they are spread out, one upon another with the flesh side up. As each skin is laid out, the flesh side is covered with a layer of common salt (sodium chloride). This is continued until a pile of suitable size is attained. The salt has a tendency to draw water from the skins, forming a brine which is generally allowed to run away. After several weeks, the skins are considered to be properly cured and they are bundled for shipment. Tanners usually have hide cellars

of their own, where they cure city butcher and country skins. Such a hide cellar is pictured in Plate 68 of Chapter 8.

### Brining.

Another method of curing, known as brining, consists in washing the skins with water, soaking them for about a day in concentrated salt solution, draining them for a few minutes, and then salting them down in piles with new salt. This is roughly the method employed for South American Frigorifico hides, noted for their good yields of leather.

### Salting vs. Brining.

McLaughlin and Theis<sup>16</sup> performed a very interesting series of experiments showing the unmistakable advantages of brining over the ordinary methods of salting. As each hide left the carcass, it was immediately split into sides. One side was salted within one hour after flaying, using fresh salt; the other was washed at once with running water, soaked for 24 hours in a 25-per cent sodium chloride solution, drained, and salted down with new salt. The treatment given left and right sides was alternated. After six to eight weeks of curing, the various lots of hides were shipped to different tanneries, the two sides of each hide going together. The lots were all worked through at once to eliminate any possible variables due to changing tannery conditions. The stock was made into vegetable tanned sole leather.

The brined hides actually produced more leather and thicker and firmer leather than the hides which were merely salted. Furthermore, the brined hides showed only about one-tenth as many so-called salt stains as the salted hides. Brining also offers a better means of standardizing the curing operation.

### Salt-Water Transfer in Curing.

McLaughlin and Theis<sup>15</sup> also made some interesting studies of the diffusion of salt into hides and of water from them during curing. They found fresh hide, as flayed, to contain about 62 per cent of water. In one test, the water content was reduced, by salting, to 41 per cent in 24 hours. 100 grams of original hide, containing 62 grams of water, had lost 35 grams of water, but had gained 6 grams of salt.

Splitting tests showed that salt is absorbed by the hide about 25 times as quickly through the flesh side as through the hair side, which is not surprising when one recalls the structure of the epidermis. It was also found that diffusion of salt into the hide is retarded by

delaying the curing after killing. A delay of one hour caused the salt to diffuse into the hide only 69 per cent as fast during the first hour as when curing was begun immediately. A delay of 6 hours reduced this diffusion rate to 26 per cent. Of course, ultimately the hides have the same salt content, but where diffusion is retarded more time is allowed for putrefactive changes which lower the leather yield. A layer of blood on the hides also retards diffusion of salt into them. Diffusion is further modified by the impurities present in the salt. Factors that retard the diffusion of salt into the hide retard correspondingly the dehydration of the hide.

### Antiseptic Value of Salt in Curing.

McLaughlin and Rockwell<sup>14</sup> have made a series of studies of the bacteriology of curing. Table XIX shows the effect of blood serum upon the concentration of sodium chloride required to stop the growth of a mixed culture of bacteria in nutrient broth incubated for 72 hours at 20° C. With no added serum, growth was stopped by 12 per cent of salt, but where 10 per cent of serum was added, the bacterial growth was not stopped by 18 per cent of salt.

TABLE XIX.

EFFECT OF CONCENTRATION OF SALT AND BLOOD SERUM UPON THE GROWTH OF A MIXED CULTURE OF BACTERIA FROM FRESH STEER IN NUTRIENT BROTH INCUBATED FOR 72 HOURS AT 20° C.

Percent NaCl in Broth	Without Blood Serum	With 3 Percent Blood Serum	With 5 Percent Blood Serum	With 10 Percent Blood Serum
2.....	+	+	+	+
6.....	+	+	+	+
10.....	+	+	+	+
12.....	—	+	+	+
14.....	—	—	+	+
18.....	—	—	±	+

The + sign indicates growth; the — sign no growth in 72 hours.

TABLE XX.

EFFECT OF CONCENTRATION OF SALT AND WHOLE CALF BLOOD UPON THE BACTERIAL COUNT OF FOUR PARTS OF BRINE AND ONE PART FRESH CALF SKIN INCUBATED 24 HOURS AND 168 HOURS AT 20° C.

Percent NaCl	Incubated 24 Hours		Incubated 168 Hours	
	No Blood	10 Percent Blood	No Blood	10 Percent Blood
0.....	68,000,000	149,000,000	1,000,000,000	2,500,000,000
2.....	14,950,000	57,000,000	260,000,000	2,200,000,000
6.....	7,900,000	15,000,000	310,000,000	633,000,000
10.....	830,000	11,900,000	179,000,000	191,000,000
14.....	60,500	470,000	33,900,000	48,000,000
18.....	37,100	650,000	8,900,000	21,000,000
25.....	21,300	50,500	70,000	310,000



Table XX shows the effect of salt and blood on the bacterial count of water used for soaking calf skin. The blood makes the bacteria much more resistant to the antiseptic action of the salt. The counts represent the number of colonies developing from one cubic centimeter of the soak water.

Even in a 30-per cent salt solution, the presence of blood had the effect of increasing the bacterial count. One part of fresh steer hide in four parts of 30-per cent salt solution was incubated for 24 hours at 22° C. Where no blood was added, the count was 630,000, but where 10 per cent of blood was added, the count was 2,380,000. McLaughlin and Rockwell point out how great may be the effect of blood in practice; when fresh steer hide is salted in the ordinary way, the brine formed during the first hour contains 40 per cent of blood, and the brine oozing from the pack after 4 weeks contains about 8 per cent.

McLaughlin and Rockwell also found, as might have been expected, that the bacterial count of soak waters at 20° C. increased as the ratio of skin to water was increased and also with the number of times the same brine was used to soak skins. They found also that salt is a better preservative of skin in weakly acid solution (pH values less than 6) than in neutral solution. This is in agreement with the findings of Merrill and Fleming given in Chapter 6; they found the danger zone of bacterial damage to lie between the pH values 6.5 and 8.0.

In an earlier paper, McLaughlin and Rockwell<sup>13</sup> have discussed the bacteriology of fresh steer hide and described bacterial cultures obtained therefrom. This bears in an interesting manner upon the problem of curing skins.

### Salt Stains.

A defect commonly found in salted skins is the appearance of peculiar stains, usually either rusty brown or greenish blue in color, which are sometimes very difficult to remove and only become intensified and darkened through contact with sulfide-lime liquors or vegetable tan liquors, substantially lowering the market value of the leather. Because they are a source of loss and annoyance to the tanner, efforts have been made, from time to time, to determine their cause and methods for preventing them. Some stains disappear when the un-haired skins are pickled with a solution of sulfuric acid and salt, but others are resistant even to this process as ordinarily conducted. These stains received the name salt stains from the general belief that they

were caused by the salt used in curing. At any rate, it was appreciated that their frequency of occurrence was influenced by the composition of the salt and the method of its application.

The percentage of stained skins was especially high in those parts of Europe where edible salt is taxed and the salt used for curing must be denatured. The use of commercial aluminum salts, particularly those containing iron, was looked upon with suspicion and the scientific men of the industry began to seek other denaturing materials that would tend to prevent rather than to cause stains.

One important school of thought regarded bacterial action as being largely responsible for the formation of the stains and sought denaturing materials capable of checking bacterial growth. Paessler<sup>19</sup> found that the percentage of stains appearing on skins could be greatly reduced by curing with salt denatured with 3 per cent of its weight of anhydrous sodium carbonate. His discovery was put into general use and had the important effect of considerably decreasing the percentage of stained skins.

Schmidt<sup>20</sup> showed that bacterial action could be effectively checked by using salt previously sprinkled with a 12-per cent solution of zinc chloride and this method has been used to some extent to prevent salt stains. But, after making a series of comparative tests, Paessler<sup>20</sup> claimed that zinc chloride was no more effective than sodium carbonate in preventing salt stains.

Romana and Baldracco<sup>27</sup> suspected the blood and lymph as the source of the stains and tried washing the skins very thoroughly after flaying and before adding the salt. On skins thoroughly washed they found no stains at all. They also found that the stains could be prevented by adding to the salt used in curing 1 per cent of its weight of sodium fluoride.

Eitner<sup>6</sup> suggested that many stains are caused by delaying the salting operation until bacterial action has already considerably advanced. He advised a more thorough elimination of water by heavily salting the skins, draining off as much brine as possible, and then resalting. The brine drained off carries with it proteins which are very susceptible to putrefaction.

Yocum<sup>41</sup> observed that salt stains occurred much more frequently in summer than in winter and were most abundant where the skins had had greatest contact with the air or had been kept for the longest period in the salted condition. Tests for iron were obtained on pieces of filter paper previously moistened with acetic acid and placed on the stains. Where stains still appeared on the finished leather, he

obtained a test for iron in the stained, but not in the unstained parts. But iron was often found in the ash of fresh skins which showed no stains when tanned at once without salting. This seemed to indicate that the staining was due to a change in the condition of the iron present which enabled it to combine with the skin. He was able to produce stains on skins by treating them with hemoglobin and suggested that the hemoglobin of the blood might have been the source of the staining material.

Becker<sup>5</sup> made extended studies of yellow, orange, and red stains on skins and isolated from them bacteria which, in pure cultures, were able to produce the corresponding stains. He also found that adding salt, up to 10 per cent of the weight of the skin, favored the action of these bacteria, while greater amounts retarded it. He warned against the use of an insufficient quantity of salt in curing, storing the skins in a warm, damp atmosphere, and of allowing dirt and filth to remain on the skins. As a means of preventing these stains, he recommended dipping the skins in a 0.25-per cent solution of mustard oil, followed by the application of plenty of clean salt denatured with sodium carbonate. Not being able to reproduce the blue stains by bacterial action alone, he admitted that these might be due to chemical changes other than those involving bacteria.

The great stress placed upon the rôle played by bacteria in the formation of salt stains adds interest to the work of Abt,<sup>1-4</sup> who maintained that most of the salt stains he had examined in France were not caused by bacterial action. Particularly bad cases of staining were traced to the presence of crystals of calcium sulfate in the salt used for curing. The stains themselves always contained considerable quantities of calcium phosphate as well as iron. The stained regions always gave more intense qualitative tests for iron than the unstained regions, but analysis showed the same actual quantity of iron in both. He pictured the stain formation as follows: Calcium sulfate present in the salt used for curing is precipitated as phosphate through contact with ammonium phosphate derived from the nucleic acids of the skin. The ammonium sulfate thus liberated then reacts with insoluble ferrous carbonate, naturally occurring in the skin, forming the soluble ferrous sulfate, which forms a stain by combining with the skin protein.

Abt attempted to follow the progress of the staining under the microscope and found that the cell nuclei disappear as the staining increases. The connective tissues gradually disintegrate, but he could find no bacteria between the altered fibers, nor did the disintegration resemble the type of decomposition produced by bacteria. He thought

the iron probably originated either in the chromatin of the cell nuclei or from the blood. A second type of stain contained no calcium phosphate, but the epithelial cells were strongly pigmented. These stains he regarded as due to the fixation of the pigment by mineral matter in such a way as to prevent its decomposition by the lime liquors later on. Abt also recommended adding sodium carbonate to salt to be used for curing because it precipitates the calcium salts present and also exerts an antiseptic and dehydrating action.

Although Abt contended that most of the stains which he had examined were not caused by bacterial action, he admitted that bacteria might play an important part in the formation of other types of stains. In fact, he <sup>2</sup> isolated an organism from one stain capable of producing a brown color on gelatin in the presence of traces of calcium phosphate and iron.

At least three different explanations have been offered to account for the effectiveness of sodium carbonate in preventing salt stains. Abt attributed it to the precipitation of calcium salts which might be present in the salt used for curing. Pacssler and others looked upon it as due to the production of an alkalinity unfavorable to the action of the bacteria thought to be responsible for the stains. Moeller,<sup>18</sup> however, suggested that the staining is a tanning action, due to such agents as the melanins or to iron and sulfur bacteria, but that this tanning action cannot proceed in alkaline solution. It is, of course, obvious that the sodium carbonate has the important effect of preventing iron salts from passing into solution, in which condition they would be free to combine with the skin forming the stains.

Summing up the work of various investigators, it would appear that salt stains are of several kinds and may be produced directly by bacteria, such as Becker's chromogenic organisms, or by soluble iron salts. These iron salts may be introduced in the salt used for curing or may be formed from the insoluble iron salts already present in the skin, either by chemical action, as described by Abt, or through the intervention of bacteria. The blood and lymph of skins furnish an excellent medium for bacterial growth and contain compounds of both iron and phosphates.

### **Ideal Curing.**

The first step in an ideal system of curing is to wash the skins free from the bulk of the blood and soluble protein matter. These materials counteract the antiseptic properties of the salt and their presence increases the dangers of bacterial damage to the skin and of salt stains.

The second step is salting. The work of McLaughlin and Theis indicates that soaking the skins over night in a 25-per cent salt solution is desirable before salting in the ordinary way. The skins should then be salted uniformly in all parts with plenty of clean salt, free from iron. Salt equal in amount to at least one-quarter of the weight of the skins should be used and any brine formed should be allowed to drain away, carrying with it any soluble proteins not previously washed out. The author believes it desirable to add to the salt used about 4 per cent of its weight of sodium carbonate. This has been known to lessen the tendency towards salt stains and it has the advantage of raising the pH value of the liquor held by the skins to about 11, which Merrill and Fleming found to be a protection against bacterial damage. During the curing, the skins should be kept in a cool place. The author has never known skins thus cured to show any of the damages ordinarily attributed to bacterial action.

#### **Action of Different Salts upon Skin Protein.**

In various experiments performed in the tannery during the past fifteen years, the author has observed the apparent destruction of both skin and fully tanned leather by certain neutral salts. For example, in a liming experiment in which the solubility of the lime was reduced by adding increasing amounts of calcium chloride, the test skins were badly damaged by high concentrations of the salt. When the concentration of ammonium chloride in a bate liquor was raised to 50 grams per liter, the skins were badly damaged. When strips of finished leather, either chrome or vegetable tanned, were soaked in concentrated solutions of calcium or magnesium chloride, they shrank to less than half their original areas.

Thomas and Foster<sup>38</sup> investigated this peculiar destructive action of certain neutral salts upon hide substance in the hope of throwing more light upon the science of curing skins. Because of the importance of their findings, their experimental procedure is given in detail.

Fifty-gram portions of American standard hide powder were covered with 1-liter portions of the chemically pure salt solutions in stoppered bottles. In view of the danger of complications through bacterial action in the dilute salt mixtures, all solutions were covered with toluene as inhibitory agent. The toluene was added to the concentrated salt solutions merely for uniformity in conditions. The bottles were stored at room temperature in the dark. They were shaken once daily during the first four weeks, and thrice or twice

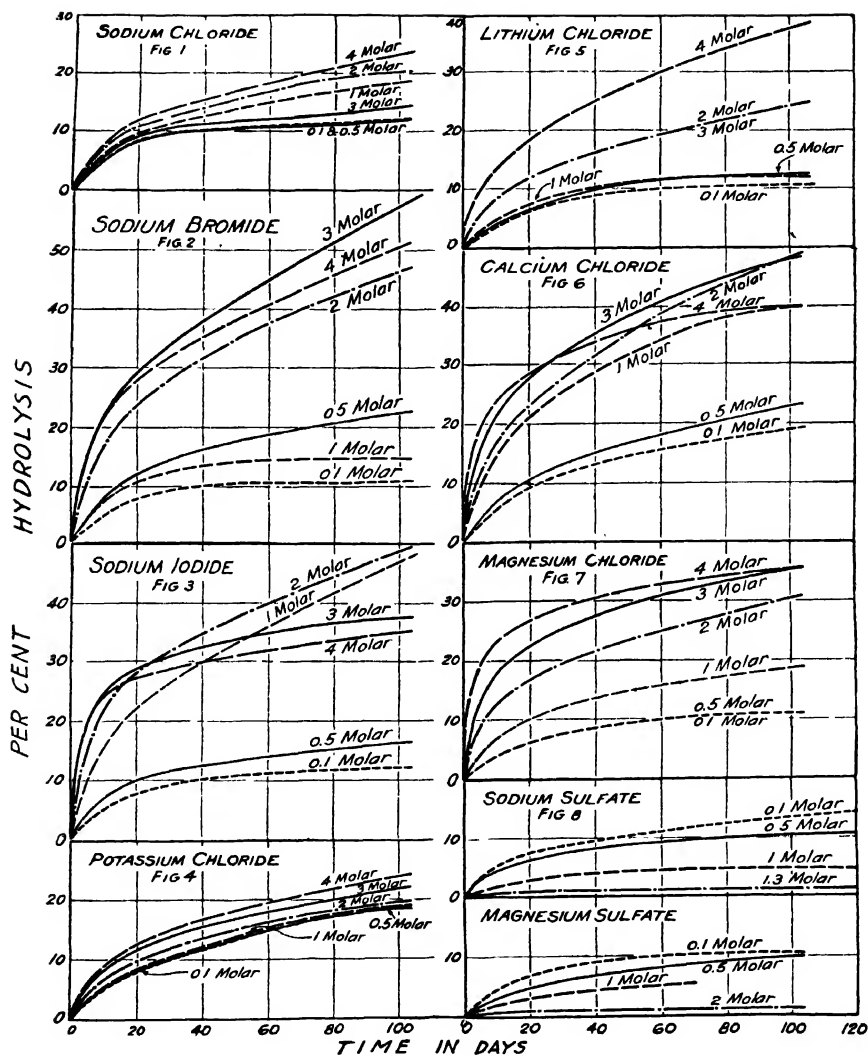


FIG. 32.—Showing hydrolysis of hide substance by solutions of various salts.

weekly thereafter. The room temperature was  $19^{\circ} \pm 2^{\circ}$  C. At the intervals of time noted in the table, specimens of the solutions were withdrawn by pipet, filtered through dry ordinary filter papers, and the filtrates subjected to the Kjeldahl process for determination of nitrogen. From the figures so obtained the percentage of hydrolysis of the hide powder was calculated. Corrections for the decrease in volume caused by these withdrawals were made in all subsequent calculations. There was none of the familiar evidence of bacterial action in any case.

The results are shown in Figs. 32, 33, and 34. The remarkable

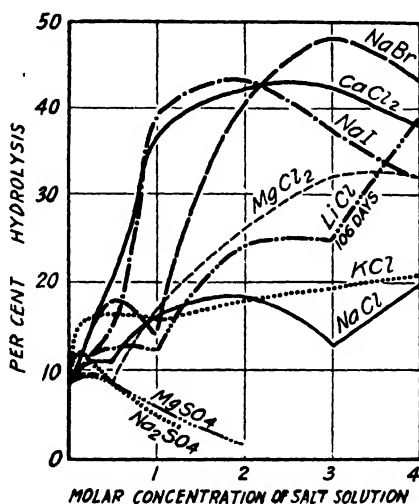


FIG. 33.—Effect of concentration of salt upon hydrolysis of hide substance.  
Time: 70 days.

finding is that all of the halides increase the hydrolysis of hide substance, while sulfates inhibit hydrolysis, making it less than in pure water. The concentration curves in Fig. 33 are curious; they were verified so as to make sure that they were not the result of any experimental error. Nor could they be attributed to variations in pH value, which was measured electrometrically. At the end of the investigation, the pH values of the most concentrated solutions were as follows: sodium chloride, 4*M*, pH = 5.1; sodium bromide, 4*M*, pH = 5.4; sodium iodide, 4*M*, pH = 6.0; potassium chloride, 4*M*, pH = 5.6; lithium chloride, 4*M*, pH = 4.9; calcium chloride, 4*M*, pH = 4.3; magnesium chloride, 4*M*, pH = 3.8; magnesium sulfate, 2*M*, pH = 4.7; sodium sulfate, 1.3*M*, pH = 5.3. It should be recalled

that the pH value of distilled water in contact with the normal carbon dioxide content of the air is 5.7.

The natural conclusion to draw from this work is that sodium sulfate is to be preferred to sodium chloride as a hide preservative. In an unpublished work, Wilson and Kern cut a number of fresh calf skins into sides and cured one side of each skin with sodium chloride and the other with sodium sulfate and stored them for about 6 months. The finished leathers were of practically identical yield and quality, indicating that the cure had been effective in both cases with a

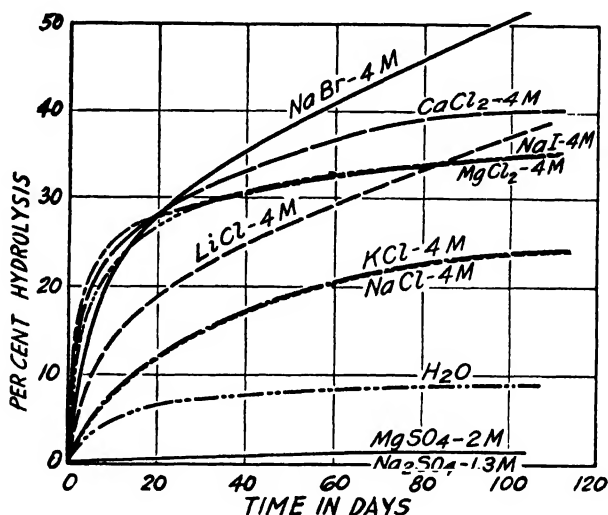


Fig. 34.—Effect of time upon hydrolysis of hide substance by various salts.

negligible degree of hydrolysis due to the salt. McLaughlin and Theis<sup>17</sup> corroborated Thomas and Foster's finding that sodium sulfate hydrolyzes hide substance to a lesser degree than sodium chloride, and found that the sulfate has a greater dehydrating action, but they criticized the suggestion that sodium sulfate be used for curing on the ground that it is not a good antiseptic; they showed that bacteria can grow in saturated sodium sulfate solutions in contact with hide substance at 20° C. We now have evidence that certain kinds of bacteria can grow on saturated solutions of either sodium chloride or sodium sulfate.

The work was pushed further by Thomas and Kelly<sup>39</sup> with very interesting results. Their general procedure was the same as that employed by Thomas and Foster, but they used toluene only on the



water control, being convinced of the practical absence of bacterial activity in the strong salt solutions employed.

Fig. 35 shows the effect of saturated salt solutions upon the hydrolysis of hide powder as a function of time and temperature.

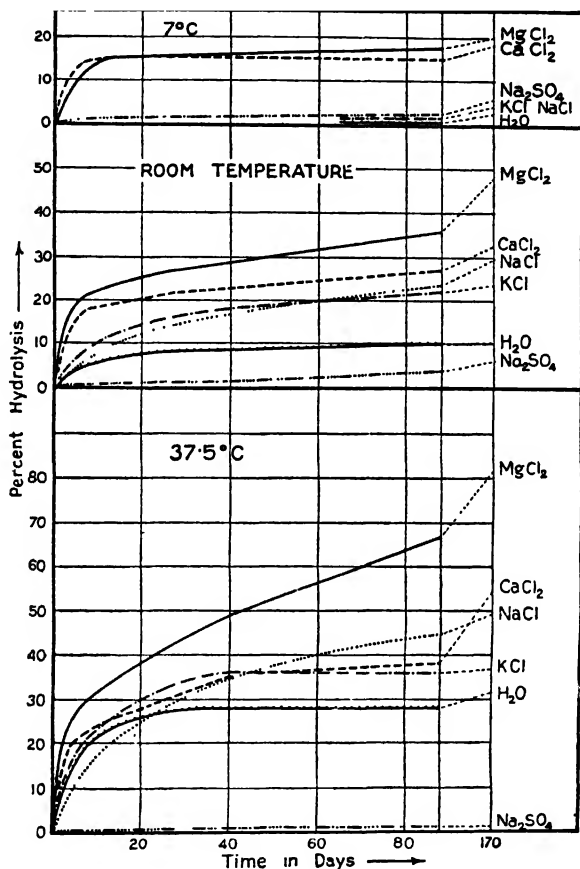


FIG. 35.—Hydrolysis of hide powder by saturated salt solutions at different temperatures.

Hydrolysis is greatly accelerated by rise of temperature for the chloride solutions or for pure water, but retarded for the sodium sulfate solution. With rise of temperature, up to 37.5° C., sodium sulfate becomes an increasingly good hide preservative. The reason for this may be found in Fig. 33, which shows that the preservative qualities of sodium sulfate increase with concentration. 100 grams of water will dissolve about 7 grams  $Na_2SO_4$  at 7° C., about 19 grams at 20° and about 50

grams at 37.5°. In contact with the warm solution of sodium sulfate, hydrolysis of the hide substance was practically zero after 170 days and no antiseptic other than the salt was used. The author has found saturated solutions of sodium sulfate at room temperature to be a better preservative of strips of skins kept in the laboratory than saturated solutions of sodium chloride.

Magnesium chloride is the most destructive of the salts tried; over 80 per cent of the hide powder was hydrolyzed at 37.5° in 170 days. The somewhat lesser action of calcium chloride is in line with the finding of Howe<sup>10</sup> that calcium chloride is a better precipitant of the proteins of blood serum than magnesium chloride.

### Effect of Salt Mixtures.

Salt used in curing is sometimes loaded with salts that retain much moisture so as to increase the apparent value of the hides, as determined by weight. It might at first be supposed that the use of calcium chloride for this purpose would result in increased hydrolysis of the hide substance, but Thomas and Kelly<sup>39</sup> made the remarkable discovery that mixtures of sodium chloride and calcium chloride exert less hydrolytic action on hide substance than either salt used alone. Saturated solutions of sodium chloride were prepared at room temperature and to each was added the required amount of a concentrated solution of calcium chloride to give the desired concentration. Since sodium chloride is precipitated from its saturated solution by calcium chloride, it was assumed that each final solution was saturated with respect to sodium chloride. The resulting solutions were analyzed for their calcium and chloride contents. The hide powder was subjected to the action of these solutions with the results pictured in Fig. 36. The compositions of the dry mixed salts ranged from 100% NaCl, 0 CaCl<sub>2</sub> to 84.8% NaCl, 15.2% CaCl<sub>2</sub> and the molar concentrations of the solutions from 5.41 molar NaCl, 0 CaCl<sub>2</sub> to 4.01 molar NaCl, 0.38 molar CaCl<sub>2</sub>.

While calcium chloride alone is more destructive in its action than sodium chloride, its addition to saturated sodium chloride solution greatly lessens its destructive action. This is an example of what the biologist calls an "antagonistic salt action." Fenn<sup>7</sup> found that the amount of alcohol required to precipitate gelatin from aqueous solution is greatly increased by the addition of either sodium or calcium chloride, but is decreased by the addition of a mixture of 100 moles of NaCl to 8 moles of CaCl<sub>2</sub>. Apparently, the presence of a

small amount of calcium chloride in salt used for curing would actually inhibit hydrolysis due to salt.

Fig. 37 shows the hydrolytic action on hide substance of saturated solutions of mixtures of sodium chloride and sodium sulfate as a

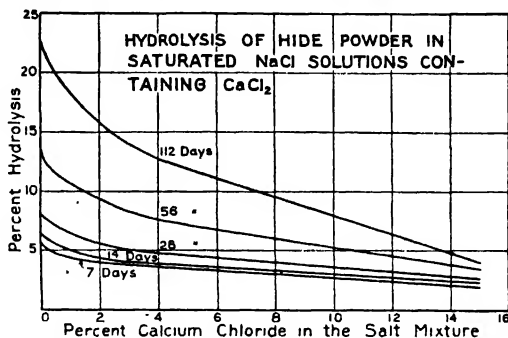


FIG. 36.—Effect of calcium chloride upon the hydrolysis of hide powder by saturated sodium chloride solutions.

function of per cent of sodium sulfate in the salt mixture and of time of contact up to about one year, at room temperature. Fig. 38 shows

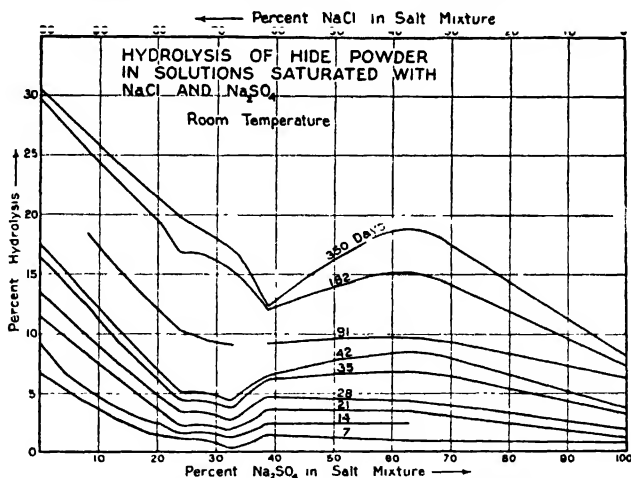


FIG. 37.—Effect of sodium sulfate upon the hydrolysis of hide powder by saturated sodium chloride solutions, at room temperature.

the action at  $37.5^\circ \text{C}$ . At room temperature, the concentration of solution varied from 5.29 molar sodium chloride and no sulfate to 1.46 molar sodium sulfate and no chloride. At  $37.5^\circ \text{C}$ . the variation was from 5.46 molar sodium chloride and no sulfate to 2.91 molar sodium sulfate and no chloride.

All mixtures of the two salts cause less hydrolytic action than sodium chloride alone, and this is especially marked at the higher temperature. In no case was any bacterial action noticeable, even after standing for a year.

Considering the extent of hydrolysis over the period 7 to 42 days, it is seen that an optimal preservative effect is obtained by using a mixture of 5 moles of sodium chloride to 1 mole of sodium sulfate. Fenn<sup>7</sup> found that the least amount of alcohol was required to precipitate gelatin from aqueous solution, in the presence of sodium chloride and sodium sulfate, when these salts were present in the molar

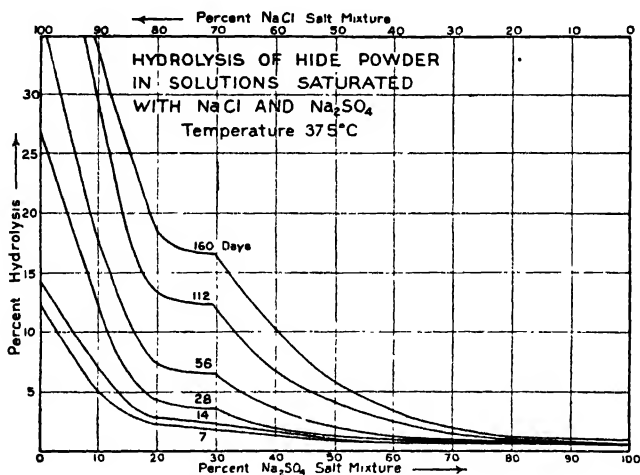


FIG. 38.—Effect of sodium sulfate upon the hydrolysis of hide powder by saturated sodium chloride solutions at 37.5° C.

ratio of 5 to 1. In this ratio, these salts exert an optimum action in tending to prevent the dispersion of hide substance or gelatin.

Fig. 39 shows the effect of pH value on the hydrolysis of hide substance by saturated solutions of sodium chloride. With increasing pH value from 0.76 to 10.0, there is an increasing degree of hydrolysis. This would seem to throw some doubt on the advisability of continuing the practice of adding sodium carbonate to the salt used in curing, but it must be considered from many different angles. Undoubtedly the chief object of curing is to decrease bacterial activity and to protect the skins against proteolytic bacteria and enzymes. In this the blood and other materials in the skin play a big part. Thomas and Kelly made their experiments with hide powder. In some of the practical tests made with different salts as curing agents, where the

kind of salt seemed to have no real influence upon the leather produced, it may be that a greater bacterial activity in one case was balanced by a greater hydrolytic activity of salt in another. The work of Thomas and his collaborators is worthy of a great deal of practical experimenting with the curing of hides with different salt mixtures.

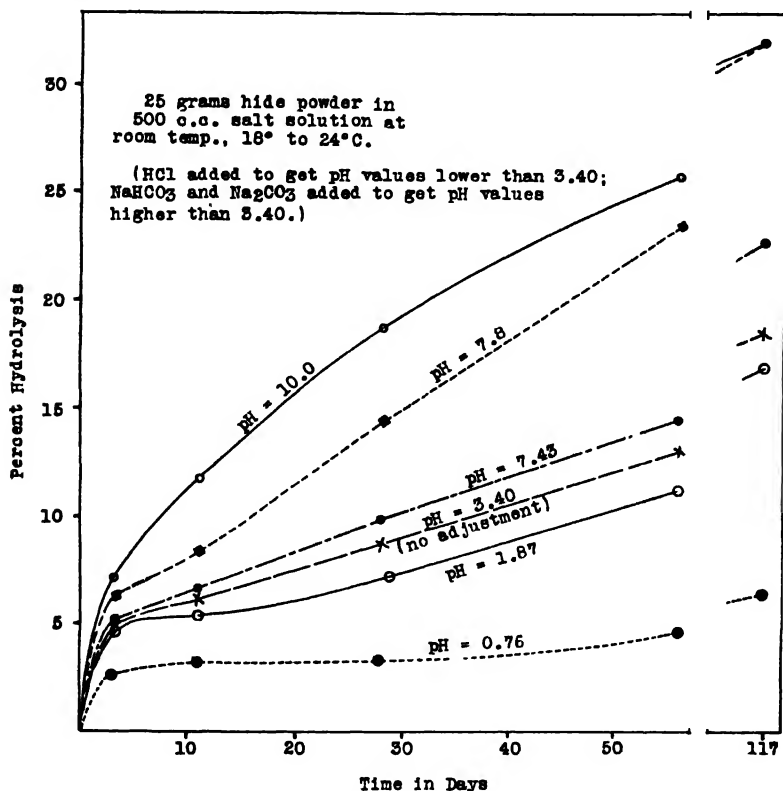


FIG. 39.—Hydrolysis of hide powder by saturated sodium chloride solutions as a function of time and pH value.

Gustavson<sup>8</sup> has offered an explanation of the action of neutral salts upon hide substance, based upon the researches of Pfeiffer,<sup>21, 22, 23</sup> Stiasny,<sup>24, 25, 26</sup> Thomas and Foster,<sup>28</sup> and others. Pfeiffer found that definite and fairly stable compounds are formed between halogen salts and amino acids, with a considerable increase in solubility and optical activity of the amino acids. The freezing point depression of the compounds formed are less than that of the sum of their components. As an example, one molecule of calcium chloride can combine

with one, two or three molecules of aminoacetic acid. Compounds of this type have been isolated in crystalline form. Similar compounds are also formed between halogen salts and the anhydrides and dianhydrides of the amino acids, including the dioxopiperazines. Secondary valency forces appear to be responsible for the combination.

According to Stiasny's theory, described in Chapter 3, the proteins consist of polypeptides held together by secondary valency forces. Gustavson thus reasons that where a neutral salt shows a tendency to break down a protein structure it first combines with the polypeptide or dioxopiperazine units. This combination not only increases the tendency for the polypeptide units to disperse by endowing them with an increased attraction for water, but it also lessens the attraction of these units for each other by using up some of the secondary valency forces which held them together. The order of the neutral salts in regard to their action on hide substance, as found by Thomas and Foster, parallels the order of simpler systems, as found by Pfeiffer. The importance of Gustavson's theory in studies of the various tannery operations will be discussed in later chapters.

The theory also suggests an explanation of the fact that a mixture of calcium chloride and sodium chloride is less destructive of skin protein than either salt used alone. These salts may form addition compounds with each other that are more stable than the addition compounds they would otherwise form with the protein. The tendency for the salts to combine with the protein, causing destruction, is thus materially reduced. A parallel case is that of the elements sodium and chlorine, which are highly reactive in certain respects when taken alone, but very much less active in the compound sodium chloride.

### **Drying.**

In tropical countries, like Java and India, from which skins are often transported very long distances, the simplest and most economical method of preserving skins is to dry them. This is true for all regions where salt and antiseptics are scarce. Moreover, drying reduces the weight of the skin by about 55 per cent. In the absence of moisture, putrefactive bacteria are practically without action on the skin proteins, although the drying does not always kill the bacteria.

When this method of preserving skins is intelligently controlled, very little damage to the skin results. In hot climates, care must be exercised to prevent excessive heating of parts of the skin which are still wet or the protein matter may decompose. Sometimes skins are

dried so rapidly that the outer layers feel quite dry, while the interior is still moist enough to permit putrefaction. Skins packed and shipped in this condition are liable to considerable damage. Defects of this kind usually cannot be detected until the tanner attempts to soak the skins back, when they may actually disintegrate or the grain and flesh layers may tend to separate, due to the hydrolysis of the protein matter in the interior. If the drying has been unduly prolonged at high temperatures, the tanner may have considerable difficulty in soaking the skins back to their normal water content.

The skin tissues continue to live for some time after the death of the animal and, in the living condition, are not readily subject to putrefaction. It is therefore desirable to dry skins as soon as possible after flaying. They should first be cleansed thoroughly by washing away all the blood and lymph and then suspended freely in a current of cool air until dry. Where conditions are such that drying cannot be effected sufficiently rapidly to prevent putrefaction, as in damp climates, it is customary to treat the skins first with naphthalene, which acts also to protect the skins against the attacks of insects during drying, but is not a powerful antiseptic.

The advantages of drying, as a means of preserving skins, are simplicity and speed of operation, independence of a supply of preservative material, and low transportation costs for the skins. The disadvantages are the difficulty of wetting the skins back later to their normal water content, the almost impossibility of detecting damage to the skin proteins until they are wet back, and the fact that dried skins may carry disease-producing bacteria or their spores in a form likely to spread infection. Dried skins do not swell in certain tannery liquors to as great an extent as fresh or salted skins, they are more resistant to hydrolysis, and they yield an appreciably firmer leather.

### Salting and Drying.

Sometimes the methods of salting and drying are combined to advantage. The skins are first salted in the usual manner, the brine is allowed to drain away, and they are then allowed to dry slowly. The salt has the effect of hindering putrefaction during the drying.

This method is extensively used in some parts of India, but the salt used is a native earth which, according to Procter,<sup>25</sup> consists chiefly of sodium sulfate mixed with sand containing insoluble compounds of iron and aluminum. This material is made into a very thin paste, which is brushed onto the flesh side of the skins. Next day more of

the paste is rubbed onto the flesh side of the outstretched skin and rubbed into it with a porous brick. After 3 or 4 saltings, the skins are dried under cover and are ready for export. The iron present in the salt sometimes causes a staining of the skins when they are kept for a long time in a moist atmosphere.

### Pickling.

Skins may be preserved by pickling in a solution of sulfuric or hydrochloric acid and sodium chloride. A solution made about N/20 as to acid and 2N as to salt is efficient. This method is not in general use for fresh skins because of the complications involved in attempting to bring them into an alkaline condition later on for unhairing. But for sheep skins, already dewooled, it is a widely used method and is convenient, because the skins are then ready for chrome tanning without further treatment.

The value of this method for preserving sheep skins is increased by the fact that wool is often more valuable than the skin. The skins are frequently purchased by *wool pullers*, who remove the wool by methods to be described in Chapter 9, and then lime, bate, and pickle them, in which condition they are stored or resold to tanners. This method of preservation permits the immediate use of the wool without destroying the skin or forcing it directly into the tanning process.

In pickling, the skins are usually thrown into a vat, equipped with a paddle wheel for keeping the liquor and skins well stirred and containing a strong solution of salt with a definite excess of sulfuric acid, which is controlled by analysis. The skins are left in the pickle liquor until equilibrium has been practically reached, which is determined by noting when there is little further decrease in concentration of acid with time. This may require anywhere from 4 to 24 hours, depending upon the thickness and condition of the skins and upon the equilibrium concentration of acid selected. Equilibrium is reached more quickly when more concentrated solutions of acid are used, but, if too strong a solution is used, it may be necessary to remove some of the acid prior to tanning by washing the skins in a concentrated neutral salt solution. After pickling, the skins are allowed to drain and are then stored in a damp condition until the tanner is ready to put them into process.

### Disinfection.

Infectious diseases among cattle are common in many countries, particularly in Asia. For this reason some kind of disinfection of



skins to be transported from infected areas is necessary in order to prevent the spread of disease germs. Much attention has been paid to preventing the spread of rinderpest, foot-and-mouth disease, and the much dreaded anthrax, which occasionally proves fatal to human beings infected with it. Various governments have issued rules to be followed in disinfecting skins from regions known to be infected. The greatest precautions have been directed against the spread of anthrax because of the danger to human life, but any treatment effective against this disease may be considered effective against the others as well.

Anthrax is the disease caused by the spore-bearing *Bacillus anthracis*. The bacillus possesses a short rod-like form and is easily destroyed. According to Seymour-Jones,<sup>28</sup> drying alone will kill the rod bacillus. The spore, on the other hand, is very resistant to methods of disinfection that do not cause some injury to the skins, and it is this that makes the problem of disinfecting skins a difficult one. Anthrax spores have been found in dried skins and in blood clots on hair and wool and, to a lesser extent, in wet salted skins.

Practical methods of disinfection are limited because so many disinfectants are injurious to the skin and reduce its value for leather making. Consequently only a few workable methods have been devised. Of these, the best known is that of Seymour-Jones,<sup>31</sup> who recommends its employment at the point of export rather than of import because of the danger of spreading the disease during transit. It consists in soaking the dried skins for from 1 to 3 days in a 1-per cent solution of formic acid containing 0.02 per cent of mercuric chloride. They are then soaked for an hour in a saturated solution of common salt, drained, and baled for shipment.

Procter and Seymour-Jones<sup>26</sup> studied the rate of absorption of formic acid and mercuric chloride during the soaking operation at a number of different concentrations, using 1 liter of solution per 100 grams of dried skin. The concentration of acid in the solution always fell slowly during a period of 20 hours, but that of the salt at first increased and then dropped, finally approaching a limiting concentration. The initial increase in concentration of mercuric chloride was found to be the result of a greater initial rate of absorption or penetration of water and acid than of the salt. The results of one of their experiments are shown in Fig. 40.

The absorption of water caused by the acid renders the skin almost as soft as in the fresh state and the subsequent immersion in saturated sodium chloride solution brings it into a condition resembling that of salted skins. Seymour-Jones points out that skins in this condition

are not only properly disinfected, but that they present less of a gamble to the tanner because they show any defects in the skin that would not be visible when the skin is in the dried state.

The effect of pH value upon the sterilizing action of mercuric chloride on coli cultures was studied by Joachimoglu.<sup>11</sup> Using one part of mercuric chloride per 600,000 of buffered solution, the sterilizing action was favored by pH values between 5.0 and 6.6, but prevented by pH values between 7.8 and 10.1.

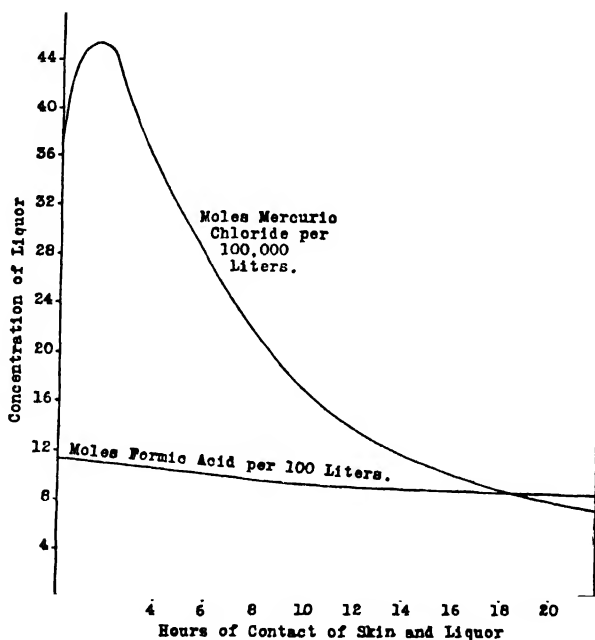


FIG. 40.—Change in composition of solution with time in the Formic-Mercury Process for sterilizing skins.

Schattenfroh<sup>28</sup> proposed a method of disinfection involving the soaking of infected skins in a solution containing 10 per cent of sodium chloride and 2 per cent of hydrochloric acid at 40° C. for 3 days. Much debate has waged over the relative merits of the Seymour-Jones and Schattenfroh methods. Tilley,<sup>40</sup> after experimenting with both methods, concluded that the Seymour-Jones process is effective, but only provided the concentration of mercuric chloride is as high as 0.04 per cent and the skins are not subjected within a week to treatment with sodium sulfide or other substance that would neutralize the disinfectant. It should, therefore, be effective where the disinfection is

carried out at a foreign port before shipping. Seymour-Jones,<sup>32</sup> in reply, pointed out that neutralization of the disinfectant by sodium sulfide would take place only in the unhairing process, whereas, under conditions existing during this process, the sodium sulfide itself is a perfect sterilizer of anthrax spores. This would seem to eliminate any possible danger of anthrax infection from skin or leather that had passed through the usual lime and sulfide method of unhairing.

Tilley found the Schattenfroh method effective when the hides were allowed to remain in the acid-salt solution for 48 hours or longer. Schnurer and Sevcik,<sup>30</sup> however, applied the Schattenfroh process to very heavy hides and obtained 4 positive tests of infection out of 11 made after the hides had been in a solution containing 2 per cent of hydrochloric acid and 10 per cent of sodium chloride for 72 hours. They attributed the more favorable results obtained by Schattenfroh to the fact that he experimented with very thin skins. Using the Seymour-Jones process on very heavy hides, they found it necessary, in order to get complete sterilization in 24 hours, to increase the concentration of mercuric chloride to 0.2 per cent, but hides so treated were found by Eitner not to have suffered for tanning purposes. They also found it necessary to degrease heavy sheep skins before applying the Seymour-Jones process, as otherwise a tenfold dose of mercuric chloride was required.

Seymour-Jones objected to the Schattenfroh method on the ground that it is workable only under laboratory conditions and that its factors of time, temperature, and general manipulation are not suited to practical operations. Ponder,<sup>24</sup> investigating methods of disinfection for the Leathersellers Company of London, and Abt,<sup>3</sup> the Pasteur Institute, Paris, working for a syndicate of French tanners, both reported in favor of the Seymour-Jones process. Apparently neither process does any injury to the skins that can be detected in the finished leather, according to the findings of numerous investigators.

Abt, however, has pointed out that hides would contain no anthrax spores, if they were dried in the sun immediately after flaying, and this view is supported by Seymour-Jones.



## Chapter 8.

### Soaking and Fleshing.

As received at the tannery, skins contain much material unsuitable for leather manufacture and which would introduce serious complications, if not removed as early in the process as possible. For this reason every effort is made to remove each undesirable constituent as soon as it can be done efficiently. The preparation of skin for tanning is carried out in a department of the tannery known as the *beamhouse* and includes, not only the removal of the undesirable parts, but also the regulation of the degree of swelling of the skin proteins.

Ears, hoofs, and tails are trimmed from skins still possessing them and the flesh, or adipose tissue, is removed by working the skin in a *fleshing machine*, which forces the flesh side of the skin against a revolving roller set with sharp blades, which cut away the adipose layer. The trimmings and fleshings make up the tannery by-product known as glue stock and are disposed of for manufacture into glue and gelatin. Plate 69 shows both a flesher and trimmer at work.

On the hair side of the skin, the epidermis is made up of a network of membranes, forming the walls of the epithelial cells, impermeable to the soluble proteins of the skin as well as to other material having large molecules or consisting of aggregates of molecules, while on the flesh side the adipose tissue consists of layers of fat cells bound together by extensive series of semipermeable membranes. It will, therefore, be readily appreciated why the adipose tissue must be removed before the skin can be thoroughly cleansed and freed from soluble protein matter.

The collagen fibers of the skin are joined together at the lower boundary of the derma in such manner as to give increased strength to the skin. In fleshing, it is important to remove all of the adipose tissue without cutting into the derma, which would weaken its structure as well as lower the leather yield. But reference to Plate 8 will show that this is not difficult where the skin is in its normal state. The lower boundary of the derma is sharply defined and the adipose tissue is not joined securely to it at all points. But where the skin has under-

gone partial or complete drying, satisfactory fleshing becomes a more difficult operation.

During the ordinary methods of drying, protein jellies suffer a change of shape, as well as of size, depending upon their initial shape, the resistance offered to shrinkage in any direction, the rate of drying, and many other factors. This was prettily illustrated by Sheppard and Elliott<sup>13</sup> with blocks of gelatin jellies. The photographs shown in Plates 64 to 67 were kindly furnished by Dr. S. E. Sheppard of the Eastman Kodak Co. Plate 65 shows four stages in the drying of a cube of 20 per cent gelatin jelly which was freely suspended in the air. No. 1 represents the original block of jelly, Nos. 2 and 3 intermediate stages in the drying, and No. 4 the dried block. At first the drying naturally proceeds most rapidly at the corners, or trihedral angles, and the faces of the cube become curved outward, as shown in No. 2, giving convex surfaces under tension. This is rapidly followed by the drying and hardening of the edges, forming a rigid framework, so that the bulk of the jelly now behaves as though suspended inside of a rigid wire frame. The faces now gradually recede and the edges become somewhat incurved until a sort of inner cube is formed with connected flanges reinforcing it, any cross-section through this having an I-beam structure, as though the drying proceeded in a manner developing the greatest resistance to stress. The flange-like edges appear to form sections of hyperboloids with a common focus at the center of the cube. Plate 64 shows three stages in the drying of a sphere of gelatin jelly. Even here the drying is not uniform, but the surface becomes puckered and wrinkled.

The dried forms of two cylinders of gelatin jelly are shown in Plate 67 and their end views in Plate 66. One base of the first and both bases of the second cylinder were allowed to adhere to rigid surfaces during the drying. The shrinkage in area of these bases being prevented, the reduction in volume had to be compensated by greater shrinkage in other directions. In the drying of a thin coat of gelatin jelly on a glass plate, the shrinkage takes place almost entirely in the direction perpendicular to the plane of the glass surface.

Upon soaking dried blocks of gelatin in water, the swelling proceeds in the direction counter to that followed during drying and the blocks tend to assume the shapes and sizes they possessed before drying.

During the drying of skin, the distortion of shape suffered by the insoluble protein constituents are further complicated by the tendency for the fibers to adhere to each other. Before a skin can be fleshed

satisfactorily, it is necessary to soak it in water long enough so that all of the insoluble protein constituents may swell to their normal sizes and shapes. When the skin is not uniformly swollen, the boundary between the derma and adipose tissue cannot be made to lie in a single plane. The fleshing machine would then cut the skin so as to leave the flesh side apparently smooth, but in so doing would either leave a considerable amount of adipose tissue on the skin to interfere with the proper cleansing of the skin or else injure the skin by cutting into the derma. The flesh side would look smooth enough upon coming from the machine, but would be ragged and irregular in thickness after the skin had been soaked further or swollen in the liquors used later. F. L. Seymour-Jones says that in Europe it is customary not to flesh skins until after at least a preliminary liming. In America, tanners of goat skins usually flesh them after liming.

Heavy, dried hides not only require a more drastic treatment than light, fresh skins, but are also better able to stand it without injury to the resulting leather. In order to get better and more uniform results, the tanner sorts the skins he receives according to weight and general condition. A suitable number of skins, all as nearly alike as possible, are assembled into a unit lot and kept together throughout the process. The treatment is then determined by the average size and condition of the skins as well as by the kind of leather desired. Very large hides are often cut into two *sides* along the line of the back bone, for convenience in handling.

Where the skins come to the tannery in a perfectly fresh condition, the soaking and fleshing operations are extremely simple. After the skins have been trimmed, the adhering blood and dirt are removed by tumbling the skins for half an hour or more in an open drum through which water is flowing. They are then fleshed, after which they are soaked in several changes of clean, cold water containing salt or a small quantity of alkali, the object of which is to free them from soluble protein matter that would otherwise contaminate the liquors used to loosen the hair and epidermis. The purpose of the salt, or alkali, is to render the globulins soluble so that they may be removed along with the albumins.

For dried, or partially dried, skins it is necessary to soak the skins both before and after the fleshing operation. The first soaking is primarily for the purpose of swelling the insoluble proteins back to their normal sizes and shapes so that the fleshing operation may be carried out efficiently. The second soaking is for the purpose of freeing the skin from soluble protein matter.

The time required for the first soaking depends upon the extent to which the skins have been dried. Completely dried skins absorb cold water extremely slowly. Since skins, as received at the tannery, are almost invariably contaminated with proteolytic bacteria, the use of warm water in soaking is somewhat risky, unless the process is very carefully watched. It is usually preferable to hasten the swelling of dried skins by adding small quantities of acid or alkali to the soak waters.

Because of the attention centered on the Seymour-Jones process of disinfecting skins, described in the preceding chapter, formic acid has often been used as a swelling agent, although other acids can be used equally as well by applying a simple system of chemical control. Alkalies, however, are more suitable where the skins are subsequently to be treated with alkaline liquors to loosen the hair. Sodium sulfide is most commonly employed to swell dried skins because it requires less careful control than the use of more caustic materials, such as sodium hydroxide. In soaking, a gallon water is usually used per pound of wet skin or for one-fifth of a pound of completely dried skin. Making the initial concentration of alkali about 0.02 normal is usually enough to initiate the swelling without causing damage either to the skin or the hair. The solution after using is then only very faintly alkaline, the greater portion of the alkali having combined with the protein matter. The alkaline liquor is used only for the first soaking after which the skins are moved into fresh water each day until swollen to normal.

Sometimes the absorption of water and softening of the skins is assisted by tumbling them in revolving drums with water between successive soakings. This is usually done with heavy, dried hides or sides.

As a rule, salted skins can be fleshed after soaking for only one day, or less. After fleshing, it has been the custom to soak the skins in successive changes of water until practically all of the salt has been removed. The salt diffuses out from the skin much more rapidly than the soluble protein matter, so that continuing the soaking until all of the salt has been removed is not unduly prolonging the process where it is desirable to free the skin as far as possible from soluble protein matter. This custom, however, has created a widespread, but erroneous, impression that it is dangerous to carry salt into the lime liquors. On the contrary, a small amount of salt assists in the unhairing and plumping of skins by the ordinary lime liquor. Its action in this respect appears to be due to the fact that it increases the hydroxide-ion concentration of alkaline solutions in general.<sup>2</sup>



### Science of Soaking.

In well established tanneries making fine leathers the operation of soaking appears extremely simple in practice, but when one attempts to work out quantitatively the optimum conditions required to produce a certain kind of leather, it takes on a complexity but little suspected from a superficial examination. The efficiency of removal of blood, coagulable proteins, and other undesirable soluble materials in skin and the absorption of water by the insoluble proteins are functions of a great many variable factors which also markedly influence the damage to skin by bacteria and enzymes and by other hydrolytic actions. Seemingly slight alterations in the procedure sometimes produce rather profound changes in the character of the leather, making it desirable in any intelligent control of soaking to study the effects of the many variables operating.

Among the most important variable factors in soaking are the constituents of the water used, ratio of skin to water, time of contact, frequency of water change, amount of agitation, temperature, pH value, salt concentration, previous history of the skins, and the kind and degree of bacterial contamination.

### Water Absorption.

One of the chief objects in soaking is to get the skin fibers to absorb as much water as they contained during the life of the animal; if they contain appreciably more or less than this amount, the skin is usually distorted in shape and lacks the suppleness characteristic of the living skin. When skins are stored, they lose variable amounts of water dependent upon the conditions of storage. McLaughlin and Theis<sup>9</sup> have demonstrated that the rate at which skins will absorb water in soaking is greatly lowered by previous drying. If the drying of a skin is not uniform over its entire area, it is difficult to correct the differences by soaking, because the regions that were wetter originally will be oversoaked before the drier regions are sufficiently soaked.

Water absorption by the skin fibers is markedly influenced by the pH value of the solution. In this connection, reference should be made to the discussion of the swelling of protein jellies in Chapter 5. Sometimes recourse is had to the addition of acid or alkali to the soak waters to hasten the water absorption of dried skins. In general, upon the addition of increasing amounts of acid, the rate of water absorption will increase until the pH value is lowered to about 2.4; any

further increase in amount of added acid or of salt will decrease the rate of water absorption. As a rule, at pH values between 2.4 and 5.0, the rate of water absorption at any given pH value will be about twice as great for monobasic acids as for dibasic acids. Upon the addition of alkali to soak waters, the rate of absorption of water by the skin fibers increases until a pH value of about 11.6 is reached, above which the rate of absorption falls off. At pH values between 8.0 and 11.6, monacid bases usually produce about twice the rate of water absorption that is produced by diacid bases at a given pH value and under conditions otherwise the same.

At the pH values 2.4 and 11.6, the skin absorbs so much water as to be excessively swollen and distorted, at least when monobasic acids or monacid bases are used. When acids or alkalies are used to hasten water absorption, it is customary to add them only in such quantity as to bring the skin to its normal water content in reasonable time. The use of greater quantities is likely to damage the skin so that, no matter how well the later processes are conducted, the final product will be below par. Safe limits may be taken as  $\text{pH} = 4$  on the acid side and  $\text{pH} = 9$  on the alkaline side, but any experiments in this field should be carried out with extreme caution, if any large number of skins is involved. For properly cured skins which have not been allowed to dry to a moisture content less than 30 per cent, it should not be necessary to add any acid or alkali to the soak water. The pH values of used soak waters, in the author's experience, usually varied from 7 to 8.

Soak waters are usually kept cold so as to retard bacterial activity, but such rises of temperature as sometimes occur in practice favor water absorption, which is also, obviously, a function of time. The kind and concentration of salt also influences water absorption, low concentrations generally favoring and high concentrations retarding it.

It is important to know when a skin has regained its normal amount of moisture. This is ordinarily determined by the feel of the skin at the time of hauling from the soaks and the accuracy of this method is repeatedly being checked by the appearance and behavior of the skins in the later operations. A properly soaked skin contains about 65 per cent of water.

### **Extraction of Nitrogenous Matter**

Another object of soaking is to remove soluble proteins that might otherwise disturb later operations. The albumins are soluble in pure water and the globulins in dilute salt solution. These proteins are

coagulated by heat, but not all of the nitrogenous matter extracted by water or dilute salt solution in soaking is coagulated by heat. From this the inference is drawn that used soak waters probably also contain degradation products of both the originally soluble and insoluble proteins of the skin.

McLaughlin and Theis<sup>10</sup> found that less uncoagulable nitrogenous matter was extracted by salt solutions than by pure water, but that the salt solution extracted a greater amount of coagulable protein. The salt apparently retarded bacterial action that was responsible for hydrolysis of the otherwise insoluble proteins and for hydrolysis also of the soluble, coagulable proteins.

Merrill<sup>11</sup> studied the effect of salt solutions upon the extraction of nitrogenous matter from fresh and cured calf skin kept under toluene to check any bacterial action. He found that increasing amounts of nitrogenous matter were extracted by salt solutions of increasing concentration, indicating that the trend in the curve obtained by McLaughlin and Theis was due to the retarding effect of salt on bacterial activity. Merrill's results are given here in some detail.

The head of a freshly flayed and fleshed calfskin was cut on the line of the backbone, and each half cut into squares about 0.1 inch edge. The material from the left half of the head was used at once; that from the right was mixed with one-fourth its own weight of salt and thoroughly dried out in the refrigerator. This cured material was then treated in the same manner as the fresh skin, which gave the desired information on the effect of curing upon the behavior of skin towards salt solution. The total nitrogen content of the fresh and of the cured skin was determined just prior to using, and samples containing the same weight of nitrogen were used in each case.

Ten-gram samples of the fresh skin, and an equivalent weight of the cured skin, were soaked with 200 cc. of the following solutions: distilled water,  $\frac{1}{8}$ ,  $\frac{1}{4}$ ,  $\frac{1}{2}$ , and 1 *N* sodium chloride. The temperature was maintained at 15° C. Each solution was covered with toluene to prevent bacterial action. After 24 hours each solution was poured through a dry filter. An aliquot of each filtrate was analyzed for nitrogen by the Kjeldahl method, and the total nitrogen that had passed into solution was calculated (as collagen). Each sample was treated with a fresh portion of solution for a second 24-hour period. The nitrogen dissolved during the second day was determined in like manner, and the process repeated until solution of nitrogenous matter had practically ceased.

The volume of each filtrate was measured, from which the volume of the solution retained by the skin sample could be estimated, and a

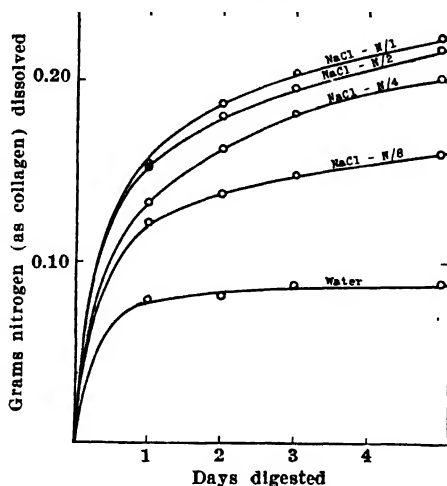


FIG. 41.—Action of salt solution on fresh calfskin.

correction was made, on all determinations after the first day, for dissolved nitrogen carried over from the preceding day.

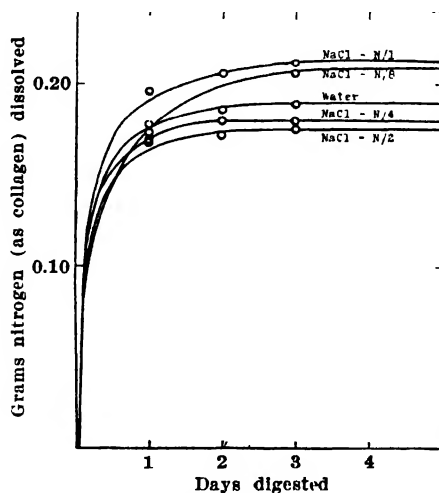


FIG. 42.—Action of salt solution on cured calfskin.

The results of these experiments are plotted in Figs. 41 and 42. The total weight of nitrogen extracted from 10 grams of fresh skin is calculated as collagen ( $N \times 5.6$ ) and plotted as a function of time of diges-

tion. In Fig. 43 final weight of extractable nitrogenous matter is plotted as a function of salt concentration.

The fact that the amount of nitrogenous matter extracted per day, after the first 5 days, became too small to measure by the method employed indicated that what was being removed was not collagen rendered soluble by the salt during the extraction period. It was probably a mixture of albumins, globulins, and other so-called soluble proteins of the skin. It is evident that cured skin differs from fresh skin in its behavior towards water and dilute sodium chloride solution.

The data for fresh skin are given in Fig. 41. The lowest curve is that obtained using distilled water. With increasing concentration of

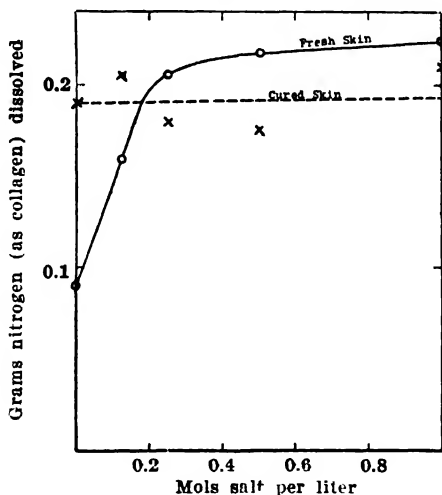


FIG. 43.—Action of salt solution on cured and fresh calfskin. Time: 5 days.

salt, the total amount of nitrogenous matter removed in any given time increases. The higher curves tend to converge, showing that the quantity of material removed approaches a limit as the salt concentration is increased. This is shown more strikingly by the continuous line in Fig. 43.

Some material is removed from fresh calf skin by salt water that is not removed by sterile distilled water and its solubility relations suggest that it consists of globulins. From 10 grams of fresh skin there was extracted about 0.09 gram of nitrogenous matter, calculated as collagen, by distilled water and about 0.23 gram by normal sodium chloride solution. The fresh skin contained 63 per cent of water, so that, of the total dry material, about 3 per cent was nitrogenous matter soluble in distilled water, 7 per cent soluble in normal sodium chloride

solution, and 4 per cent soluble in the salt solution, but insoluble in distilled water.

As will be seen from Fig. 42, very little nitrogenous matter is extracted from the cured skin after the first day. The most striking difference between these results and those obtained with fresh skin is that salt concentration has but little effect upon the quantity of nitrogenous matter extracted. In Fig. 42 the order of the curves from the X axis is not related in any rational way to the salt concentration and in Fig. 43 we see that the quantity of material extracted is independent of the normality of the salt, within the limits of experimental error, which here is rather large. Just as much nitrogen is removed by water as by normal sodium chloride solution. The quantity of material removed from cured skin by salt water is, on the other hand, slightly less than that removed from fresh skin.

In seeking the reason for this difference in the behavior of fresh and cured skin, it must be remembered that the cured skin, when soaked, carries a certain amount of salt into solution. For this reason the salt concentrations used for the first, but not for the subsequent, soakings are all somewhat higher than those indicated. This is not sufficient, however, to explain the greater extractive action of the weaker solutions upon the cured skin. Of greater importance, probably, is the fact that during the curing the skin proteins are subjected to the action of saturated brine. This treatment probably renders soluble in water any material that otherwise is dissolved only by strong salt solutions.

An alternative explanation lies in the effect of bacterial action on the cured skin. Despite salting, drying, refrigeration, or all three combined, considerable bacterial action goes on in a skin during curing. This results in the production of a large amount of water-soluble nitrogenous matter. The irregularity of the data obtained with cured skin is in point here, for if much of the water-soluble material in cured skin is due to bacterial damage it is easy to see that irregularities might creep in, due to unequal damage to different parts of the material used for the experiments. The decrease in salt-soluble material brought about by curing is probably due to coagulation of a portion of the globulins on drying out.

The scientific value of Merrill's work lies in the elimination of the variable factor of bacterial activity during the soaking so as to indicate the effect due to salt alone, but these sterile conditions do not obtain in practice. Working under tannery conditions, it is not easy to differentiate causes and effects. A change in pH value, salt concentration, or temperature of the soak water may produce a change in the kind and

amount of nitrogenous matter extracted, but the effect may be produced directly on the skin constituents or it may produce the visible effect through a modification of bacterial activity.

Working under practical conditions, McLaughlin and Theis<sup>8, 9, 10</sup> have studied the effect of time, temperature, salt concentration, pH value and ratio of water to skin in soaking. For details, their papers should be consulted.

### Bacterial Action

The commonest source of danger in soaking is bacterial action. Although the inner surface of the skin on the living animal may be free from bacteria, it acquires them from the atmosphere very rapidly from the instant of flaying and acts as an ideal medium for the reproduction of bacteria. By the time the skin reaches the soak vats, it is usually contaminated with countless millions of bacteria. Many species of these bacteria are known to secrete enzymes that hydrolyze collagen. The chief practical object to be gained from a study of the bacteria common to tannery soak waters is to find means of destroying them, or at least of preventing them from doing any damage to the skins.

Andreasch<sup>1</sup> isolated a number of species of bacteria from tannery soak liquors of which he identified the following:

- Bacillus fluorescens liquefaciens* (Flügge).
- B. megaterium* (de Bary).
- B. subtilis*.
- B. mesentericus vulgatus*.
- B. mesentericus fuscus*.
- B. mycoides* (Flügge).
- B. liquidus* (Frankland).
- B. gasoformans* (Eisenberg).
- White bacillus (Maschek).
- Proteus vulgaris*.
- Proteus mirabilis*.
- B. butyricus* (Hueppe).
- White streptococcus (Maschek).
- Worm-shaped streptococcus (Maschek).
- Grey coccus (Maschek).

All these may be classed as putrefactive organisms that secrete a variety of enzymes, many of which act energetically on hide substance.

Rideal and Orchard<sup>12</sup> examined the action of *B. fluorescens liquefaciens* on gelatin to which had been added 10 per cent of Pasteur's

solution to serve as nutrient medium. The gelatin was completely liquefied in three and one-half days. It was shown that the liquefaction of the gelatin was due to an enzyme secreted by the bacteria. The liquefied gelatin was alkaline and had a slight odor suggesting putrefaction, but contained no hydrogen sulfide. A notable feature was the small amount of ammonia and volatile bases produced; only 0.2 gram of ammonia per 100 cubic centimeters was produced even after 16 days' incubation.

Wood has done a great deal of pioneer work in the study of bacteria and enzymes in tannery liquors. Plate 72, taken from one of Wood's <sup>18</sup> papers, shows a typical plate culture on gelatin of a soak water used for softening dried sheep skins, in which no chemicals were used. The development of the colonies had to be stopped by the application of formaline vapor before many of the species had time to develop; otherwise the whole plate would have been liquefied.

McLaughlin and Rockwell <sup>5</sup> made an interesting study of the microorganisms found on fresh steer hide. They identified cocci, staphylococci, streptococci, large and small bacilli, which were motile and non-motile and gram positive and gram negative, spirilla, spirochaeta, yeasts, oïdia, molds, and protozoa. From these, they selected for special study the 24 strains of bacteria which predominated. They appreciated, of course, that to isolate and catalog all of the bacteria that one might find on skins or in tannery liquors would be an endless and probably useless task. It did seem worth while, however, to divide them into proteolytic and non-proteolytic groups and to study the action of each group on skin.

They found that the factors which favor decomposition of skin by bacteria are the presence of proteolytic bacteria, protein substances such as blood, a slightly alkaline reaction, warm temperature, and the presence of oxygen and small traces of carbon dioxide. Decomposition was hindered by the absence of proteolytic bacteria, the presence of non-proteolytic bacteria, acidity, fermentable carbohydrates, absence of protein material, low temperature, absence of oxygen, and the presence of much carbon dioxide.

Under favorable conditions, the rate of growth of colonies of bacteria is a logarithmic function of the time. Thus in a soak water the number of bacteria present increases more and more rapidly until dangerous numbers are reached. Plotting the growth as a function of time, a curve is obtained which rises very slowly at first and then with enormously increasing rapidity. The part of the curve from the origin to the point where the upturn becomes marked is known as the lag



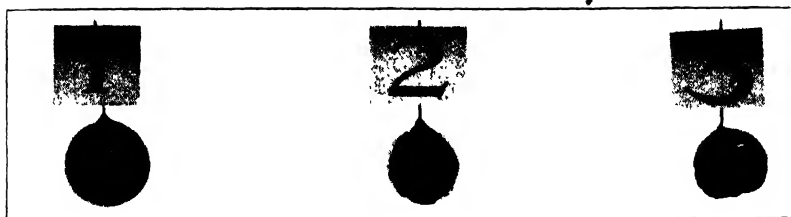


Plate 64.—Three Stages in the Drying of a Sphere of Gelatin Jelly.

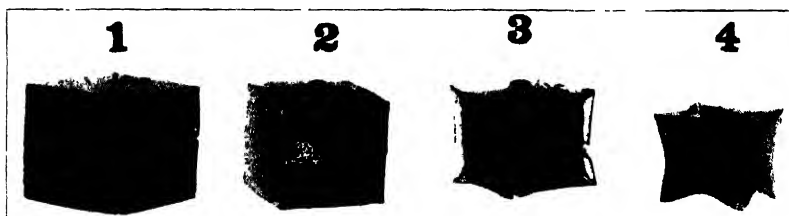


Plate 65.—Four Stages in the Drying of a Cube of Gelatin Jelly.



Plate 66.—End Views of Dried Cylinders of Gelatin Jelly.

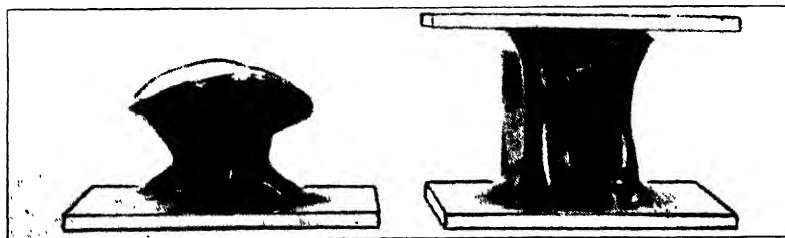


Plate 67.—Two Cylinders of Gelatin Jelly Dried with One and Two Faces, Respectively, Adhering to Rigid Surfaces.



Plate 68.—Salting and Trimming.

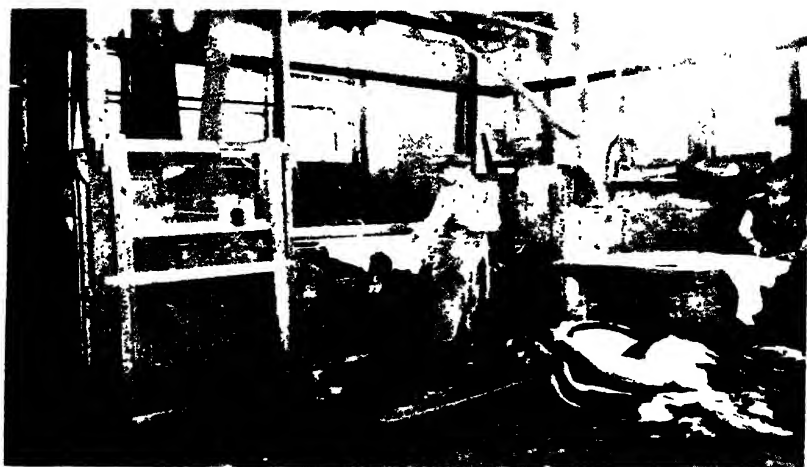


Plate 69.—Fleshing and Trimming.



Plate 70.—Analytic Laboratory of Modern Tannery.



Plate 71.—Laboratory Experimental Tannery.



Plate 72.—Typical Plate Culture on Gelatin of Soak Water Used for Softening Dried Sheep Skins.

period. McLaughlin and Rockwell<sup>7</sup> point out that the maximum period of soaking should never be longer than the minimum lag period. In soaking calf skins with 1 part of skin to 4 parts of water, they found a lag period of 36 hours at 10° C., 18 hours at 15°, 10 hours at 20°, 4 hours at 25°, and only 2 hours at 30°. The lag period decreased as the proportion of water to skin was raised from 10 hours with 4 to 1 down to 6 hours when there was 10 parts of water to 1 of skin. The lag period increased with increasing concentration of salt.

### Antiseptics

The bacterial activity of soak waters can be reduced to a negligible degree by the addition of antiseptics. If the skins are washed in running water before entering the soak vats and are given a sufficient number of changes of clean, cold water, the use of antiseptics is quite unnecessary, but it is not always practical to soak in cold water.

The author has made a study of the effect of various antiseptics on the bacterial count of tannery soak waters. In these tests calf skins were soaked in paddle vats in the ratio of one pound of skin to 8 lbs. of water. The water used was a sterile well water having a temperature of 12° C., a pH value of 7.8, an alkalinity of 250 parts per million as calcium carbonate, and other constituents, in parts per million, as follows: CaO 72, MgO 5.0, Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> 3, SO<sub>3</sub> 66, Cl 11, and SiO<sub>2</sub> 8. The water was changed once every 24 hours for 3 days. On an average, the bacterial count in the incubator at 20° C. of the first soak water was 1,000,000 per cubic centimeter, of the second soak water about 2,000,000 and of the third soak water about 4,000,00. It may seem strange that the count should increase when the water was becoming cleaner through successive changes, but such was the case. The temperature rise during the 24 hour soaking period in each case averaged only 2°. The bacterial count was increased by excessive paddling, probably due to breaking up large colonies into smaller ones. Where no antiseptic was added, the final pH value of the water averaged 7.4. This is the pH value at which bacterial activity is greatest, according to the findings of Merrill and Fleming, described in Chapter 6.

Of all the antiseptics tried, the most economical was chlorine. This was introduced into the water line running to the soak vats by means of a Wallace and Tiernan chlorinator,\* such as is widely used in chlorinating municipal water supplies. In each test 700 skins from the same source were divided into two lots of 350 each and one lot was soaked only in plain well water and the other in chlorinated water.

\* Wallace and Tiernan, Newark, N. J.

Where no chlorine was used, the counts after the first, second and third days' soakings were 873,000, 2,240,000, and 3,496,000, respectively. Where water containing 10 parts per million of chlorine was used, the counts were 43,000, 191,000, and 372,000, respectively, indicating a reduction of 95% in the first soak water, 91% in the second, and 89% in the third, brought about by the use of 10 parts of chlorine per million of water. The final pH values in all cases lay between 6.9 and 7.5, the effect of the chlorine in this respect being too small to measure.

The yield and quality of the finished leather in all comparison lots was the same, whether chlorine had been used in the soaks or not. This was taken to indicate that the bacteria were doing no harm in those vats where chlorine was not used, which may be attributed to the low temperature and general cleanliness of the operations. In one test the temperature was allowed to rise to 25° C. and to remain there during the 3 days of soaking, with the result that the skins suffered irreparable damage, being both loose in structure and spotted when finished. It is reasonable to believe that the use of chlorine is advantageous where conditions are such that the bacteria will do damage, as where only warm water is available for soaking.

Adding chlorine to the first soak water only did not result in any lowering of the count on the second and third waters, where these contained no added chlorine. It was found necessary to add chlorine to all of the water used in order to keep down the count.

Sulfur dioxide lowered the bacterial count appreciably only when used in such quantity that it produced a permanent roughening of the grain surface. Sodium fluoride seemed to increase the count, but it was discovered that this was a salt effect upon the method of making bacterial counts; this is discussed in Chapter 6. In order to get a 95% reduction in bacterial count of the first soak water with mercuric chloride, it was necessary to use 50 parts per million, indicating that it is only one-fifth as powerful as chlorine by weight. Saturating the soak waters with lime proved to be about as effective as the use of 10 parts per million of chlorine.

Para-chlor-meta-cresol has been used as an antiseptic in soaking, apparently with success. The use of 500 parts per million proved effective under conditions such that skins soaked without this material were badly damaged. Stiasny<sup>14</sup> has recommended the use of zinc chloride in warm soaks or for soaking skins which have not been properly preserved. He finds that the use of 1000 parts per million will permit leaving the skins in the soak water for six weeks or more without



## Chapter 9.

### Unhairing and Scudding.

After the skins have been trimmed, cleansed, freed from adipose tissue and soluble matter, and have again become soft through absorption of their normal water content, they are ready for the series of operations involved in the removal of the epidermal system. It will be recalled from Chapter 2 that this system includes the epidermis, hair, and the sebaceous and sudoriferous glands and differs from the true skin under it in origin, structure, method of growth, and chemical composition. The several parts of the epidermal system differ markedly in their resistance to chemical reagents and it is rather fortunate for the tanner that the part most readily digested is the portion of the Malpighian layer resting on the grain surface. When the epithelial cells of this layer are destroyed, the rest of the epidermis and the hair become completely separated from the true skin and can easily be removed mechanically.

#### Sweating

What is probably the oldest method known for unhairing skins received the name *sweating* from the nature of the process in its more highly developed state. It consists of little more than the putrefaction of the cells of the Malpighian layer. Since it is only necessary to allow a fresh skin to remain for a day or two in a warm, damp place to cause a loosening of the hair, the method was probably discovered very early in the history of the human race. It is not improbable that the accidental discovery of this action first revealed to the ancients the advantages of unhaird skins for certain purposes.

Because of the danger of serious damage to the skins in the sweat chambers, unless the process was very carefully watched and controlled, it ceased to be popular for the best grades of skins after safer methods of unhairing were devised. It is still in use in some tanneries for the lower grades of skins, such as the cheaper classes of dried hides and sheep skins where the wool is valued more highly than the skin.

The skins are generally hung from beams in a closed room in which



the air is kept warm and humid. The temperature, humidity, and ventilation must be carefully controlled. During the process a considerable quantity of ammonia and amines are evolved and these assist in the unhairing action. Just as soon as the hair slips easily, the skins are removed from the sweat chamber and dumped into saturated lime water. The lime water serves to retard further bacterial action and to cause the skins to swell somewhat by absorption of water; the skins upon coming from the sweat chamber are in a very flaccid and slimy condition.

Wilson and Daub<sup>33</sup> made a study of the sweating process under the microscope. Pieces of fresh sheep skin were kept in a closed receptacle having an atmosphere saturated with water vapor at 38° C. At frequent intervals strips of skin were removed for sectioning and examining under the microscope. At the end of 42 hours, the wool could be rubbed off with ease and the skin had apparently suffered no damage. The odor of ammonia in the receptacle after the first day was very pronounced.

The first sign of action visible under the microscope was the separation of the cells of the Malpighian layer from one another and from the surface of the derma. This action gradually spread to the outermost layers of cells of the sebaceous and sudoriferous glands. On the second day the action had proceeded so far that the epidermis, glands and wool were completely separated from the derma and many of the epithelial cells had completely disintegrated. A section of the skin after being in the sweat chamber for 42 hours is shown in Plate 75. The upper portion of the section is shown in Plate 76 at a much higher magnification.

It will be noted that the corneous layer is still intact, but the Malpighian layer has almost completely disintegrated, the linings of the hair follicles are broken up, and the glands have all been loosened and separated from the derma. Plate 75 should be compared with Plate 30, which represents a section from the same skin fixed in Erlicki's fluid within an hour after the death of the animal.

In practice, the systematic cleaning of the sweat chambers is necessary in order to prevent the increase of undesirable organisms that may be carried in from time to time. Hampshire<sup>8</sup> investigated the cause of a pitting, or liquefaction in spots, of the grain and flesh surfaces of sheep skins, a damage known to the trade as *run pelts*. He found that the pittings was caused by several species of wormlike organisms belonging to the family *Nemathelminthes* and growing to a length of about one millimeter. Apparently they are killed by simple drying.

They were found in great numbers in the sweat chambers, but not on skins which had not yet entered the chambers. In laboratory experiments, they produced a pitting of the skin in the presence of a small amount of ammonia, such as is always present in the sweat chambers. It was found that uniform slipping of the wool could be produced by incubating the skin in a clean vessel which excluded all organisms other than those present on the incoming skin, and skin treated in this way was free from pitting. It would seem that the danger of run pelts can be completely avoided by making certain of the cleanliness of the sweat chamber before the skins enter.

Upon coming from the sweat chamber, the skins are usually put into saturated lime water, which checks the bacterial action. After the skins have been in the lime water for from several hours to a day, they are hauled out to be unhaired. The operations from this point on are essentially the same as when the hair is loosened by means of lime and will be described in connection with liming.

### Liming

The commonest method in use today for effecting the separation of the epidermal system from the true skin is also one of ancient origin and is known as liming from the fact that saturated lime water is used. Formerly a lime liquor was prepared simply by filling a vat with water and adding calcium hydroxide greatly in excess of saturation. The skins, after soaking, were put into this liquor and allowed to remain there until the hair and epidermis had become so loosened that they could be rubbed off with very little pressure. Often the skins were removed each day and fresh lime added in order to hasten the action. But with a fresh lime liquor it usually required a week or longer for the skins to get into a state where the hair would slip easily. It was discovered that less time was required for each succeeding lot of skins passing through a given liquor. The longer a liquor was used the more it became charged with ammonia, amines and other protein decomposition products, bacteria, and enzymes, the actions of which have been the subject of much study. The older liquors also produced less swelling of the skin proteins than fresh liquors.

As more was learned of the action of lime liquors, it became customary to employ a series of liquors for each lot of skins. The skins were put first into the oldest liquor in order to start the loosening of the hair. Each day they were moved into a fresher liquor and finally into one quite fresh. This system is still in use in many heavy leather

tanneries, but the modern tendency in light leather tanneries is toward quicker methods.

When lime alone was used in making lime liquors, it usually required about a week or more to cause the hair to slip easily, depending upon the temperature, during which time a considerable amount of collagen became hydrolyzed, especially in old liquors or in liquors not kept completely saturated with lime at all times. Bacteria are very sensitive to changes in pH value and many proteolytic bacteria present in lime liquors which are comparatively inactive at a pH value of 12.5, that of an ordinary lime liquor, become very active as the pH value falls to lower values. In order to guard against the danger of incomplete saturation of the liquors with lime, mechanical agitators have been devised, one of the simplest being a paddle wheel set in the vat, like that shown in Plate 73. By keeping the undissolved lime continually stirred up, the solution is kept almost at the saturation point.

With increasing demand for speed of operation and conservation of the skin collagen, *sharpening agents* have come into wide use, the principal ones being arsenic sulfide, sodium sulfide, and sodium hydroxide. The judicious use of these materials, in conjunction with lime, has reduced the time required to unhair skins materially. More attention was paid also to temperature. In some of the old tanneries not equipped to heat the liquors, a much longer time had to be allowed for unhairing in winter than in summer. It is now customary to maintain a uniform temperature of from 20° to 25° C. the year round.

Arsenic disulfide was one of the first sharpening agents to be employed. It was mixed with the lime before slaking in the proportion of about one part of sulfide to twenty-five parts of lime and from this mixture a liquor was made of such concentration that the hair would not be damaged, but would slip easily in two or three days. Sodium sulfide is now used more commonly than arsenic, being cheaper and somewhat more effective in loosening the hair. It is used at about 0.01 molar concentration in a solution kept saturated with lime.

The action of a lime liquor sharpened with sodium sulfide upon a calf skin is illustrated in Plate 77. A fresh calf skin was put into a solution containing 0.7 gram of  $\text{Na}_2\text{S}$  per liter and calcium hydroxide well in excess of saturation. The liquor was agitated frequently and kept at a temperature of 25° C. Strips of the skin were examined at intervals as in the study of the sweating process. The skin from the sweating process was in a soft, flaccid condition, while that from the lime liquor was plump and rubbery, but the fate of the epithelial cells of the Malpighian layer was the same in both cases. Sections of speci-

mens taken at intervals showed these cells slowly disintegrating and leaving the corneous layer, hairs, and glands separated from the derma. Plate 77 shows a section taken after the skin had been in the lime liquor for 48 hours. Part of the upper region of the section is shown at higher magnification in Plate 78. The section is from the same skin as that shown in Plate 28, which represents the fresh skin as it existed in life.

The lime has completely destroyed the Malpighian layer of the epidermis and the corneous layer appears as a nearly continuous line somewhat separated from the true skin. The epithelial cells of the hair follicles have been completely broken up, leaving the hair, with adhering patches of corneous layer, free to be swept out by the action of the unhairing machine. The sudoriferous glands have disintegrated, leaving empty spaces, and the sebaceous glands may be seen lodged in pockets opening into the hair follicles. The erector pili muscles are still intact and can be seen running upward to the left from the region of the hair bulbs. In the thermostat layer, as well as in the deepest layer of the skin, the elastin fibers appear as fine dark threads.

Although the hair loosening operation can be effected easily in a single liquor acting for one to three days, some tanners still prefer to use a series of liquors, claiming that they get a result better adapted for the particular kinds of leather they desire to make. They lessen the extra amount of labor involved in handling the skins by a system of reeling from vat to vat. The skins are all hooked or tied together, the head of one to the tail of another, and the whole lot is passed over a reel from one vat to another, the last skin in being the first to come out. The skins are put first into the oldest liquor and then reeled into a fresher liquor each day until ready to be unhaired.

When the skins have been in the lime liquor until the epidermis has become completely loosened, they are hauled out and the hair and epidermis are removed on an unhairing machine. Plate 73 shows a laborer hauling skins from a paddle vat of lime liquor. Plate 86 shows the skins being unhaired on a machine. The skins are thrown over rubber slabs and forced against a roller set with blunt knife blades which rub off the loose hair and epidermis. In the machine shown in the picture, the slabs containing the skins move in a Ferris-wheel motion, brushing against the roller which can be seen in the lower part of the machine. There are many types of unhairing machine in use, but most of them depend upon the principle of scraping the hair surface of the skin with dull knife blades.

Each skin is next placed over a *beam* and *scudded*. The beam, from which the beamhouse derived its name, is a convex wooden slab sloping upward from the floor, at an angle of about  $30^\circ$ , to a point about three feet high, which gives it a length of about six feet. The *beamster*, leaning over the beam, pushes a specially designed, two-handled knife over the skin downward and to left and right, forcing the remnants of the glands, lime soaps, dirt, and any remaining hairs out of the hair follicles and pores. This operation is known as scudding. Plate 74 shows a group of beamsters scudding calf skins.

By carrying the destruction of the epidermis and hair far enough, it is possible to get the skin into condition to permit mechanical scudding on a machine operating like an unhairing machine, but it is not always desirable to do this. Scudding can usually be done better by hand than by machine because the hair follicles slope in many different directions. If the knife stroke is made in the direction of the hair, from root to tip, the dirt in the follicles is easily squeezed out, whereas there is a tendency for it to be trapped by a stroke in the opposite direction. There is a sufficient degree of transparency to a limed skin to enable the beamster to see the dirt and pigment in the follicles and he directs his knife first one way and then another until the skin appears clean. He is also on the lookout for fine hairs not removed by the machine. The bulb of a new hair is as deeply seated as that of an old one, but there may not be enough of the new hair protruding above the surface of the skin to be gripped by the knives of the unhairing machine.

After the scudding operation, the skins are washed thoroughly to remove as much lime as possible. This washing is of considerable importance because any great excess of lime carried forward interferes with the later processes. It is customary to wash the skins in a revolving drum through which fresh water is continually passing. Wood<sup>37</sup> followed the removal of lime during washing and showed that little is to be gained by continuing the washing for more than two hours. The tendency, however, is to wash the skins for a shorter time than this and to take care of the residual lime by other means. Fig. 44 shows the extent of lime removal with time during a typical washing operation. The lime left in the skins appears to approach a limiting value, due to the lime which has carbonated as well as that in chemical combination with the skin.

Studies of liming are greatly complicated by the large number of interrelated variable factors involved, to appreciate which one has only to recall the complex structure and chemical composition of the skin

and then try to calculate the probable effect on each individual constituent of variations in pH value, temperature, time of contact of skin

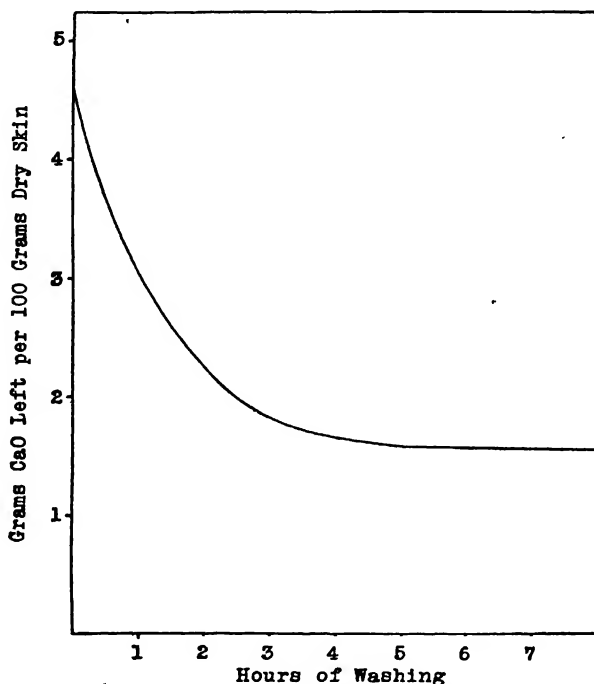


FIG. 44.—Removal of lime from unhaird skin by washing.

and lime liquor, and kind and concentration of salts, protein decomposition products, enzymes, and bacteria.

### Plumping and Falling

When animal skin is immersed in dilute solutions of acid or alkali, the protein matter swells by absorbing some of the solution, but the effect to a casual observer is not so much one of swelling as of increased resiliency of the skin, due to its fibrous structure. The collagen fibers, in swelling, tend to fill up the interstices between them and the full increase in volume of the protein matter is not evident from the appearance of the skin. A skin in which the fibers are not swollen may contain practically as much water as one whose fibers are swollen, as in lime water, but the bulk of the water in the first skin is held only

loosely *between* the fibers and may be squeezed out by the application of slight pressure, whereas that in the second is present *within* the substance of the fibers, like the water absorbed by a solid block of gelatin jelly, and cannot be removed, except by the application of enormous forces. During the swelling of the protein matter, the tanner observes in the skin an increasing resistance to compression, to which he has given the name *plumping*, the term *falling* indicating the reverse action.

Wood, Sand and Law<sup>39</sup> devised an apparatus for determining when a skin had become completely fallen during the bating process which consisted of a sensitive thickness gauge in which the pressure exerted upon 1 square centimeter of skin could be varied by means of weights. The point of complete falling of a skin was taken as that at which no recovery in thickness of the skin took place upon removing the weights. The apparatus was also used to measure the apparent modulus of elasticity of the skin and this was considered to be a measure of the degree of plumping.

This method suggested to Wilson and Gallun<sup>40</sup> another which is more suitable for certain purposes. Their apparatus consisted of a Randall and Stickney thickness gauge\* with a flat, metal base upon which a small piece of skin could be placed, and a plunger, having a circular base 1 square centimeter in area, capable of pressing on the surface of the skin under constant pressure. The apparent thickness of the skin, as shown on the dial of the instrument, being determined by the position of the plunger, decreased with time as the plunger caused an increasing degree of compression. For this reason and in order to get comparative readings, all gauge readings were taken a fixed length of time after dropping the plunger onto the skin. In order to measure the degree of plumping of skin in a given liquor under fixed conditions, they first measured the resistance to compression of a small piece of skin under standard conditions. This same piece of skin was then subjected to the conditions of the test and its resistance to compression measured again. In each case the gauge reading was taken as a measure of the resistance to compression. The ratio of the final to the initial gauge reading is a measure of the degree of plumping of the skin. Their measurements of the degree of plumping of calf skin as a function of pH value are given in Chapter 10.

If a skin in the alkaline state is pumped or swollen excessively, it suffers permanent distortion because all of the protein constituents of the skin do not swell equally and the value of the final leather is

\* Randall and Stickney. Waltham, Mass.

lowered. Some knowledge of the degree of plumping of skin in liquors used for unhairing is therefore much to be desired.

Atkin<sup>1</sup> was able to reason from the work of Procter, Wilson, and Loeb, which was discussed in Chapter 5 in connection with the swelling of protein jellies, that arsenic disulfide is preferable to sodium sulfide for certain kinds of skin where fineness of grain surface is of paramount importance in the finished leather. Loeb showed that diacid bases produce a maximum swelling of gelatin jelly only half as great as that produced by monacid bases. Atkin confirmed this for the swelling of hide powder and showed that the weak base ammonium hydroxide produces as much swelling as sodium hydroxide at the same pH values. When arsenic disulfide is slaked with lime and used in a fresh liquor, the solute consists only of calcium hydroxide, calcium sulfhydrate, and calcium sulfarsenite. But when sodium sulfide is used as the sharpening agent for a lime liquor, sodium hydroxide and sodium sulfhydrate are present. It would therefore be expected that the use of sodium sulfide would result in a greater plumping of the skin than the use of arsenic sulfide, which gives a liquor containing only divalent cations. In actual practice, when arsenic sulfide is used to sharpen lime liquors for the unhairing of goat skins in the manufacture of glazed kid leather the final leather has a smoother and silkier grain surface than when sodium sulfide is used in the lime liquors. This may not be due entirely to the presence of only divalent cations, however, because any decrease in solubility of calcium hydroxide brought about by the presence of calcium sulfhydrate or other calcium salt would lower the pH value of the liquor and the swelling of the skin accordingly.

Stiasny and Wurttenberger<sup>20</sup> consider calcium sulfhydrate to be the active agent in arsenic limes and recommend that they be so prepared as to give a maximum yield of this substance, which is favored by a fine degree of subdivision of the lime and arsenic disulfide, absence of arsenic pentasulfide or oxides of arsenic, and low temperature. Atkin, Watson and Knowles<sup>3</sup> recommend treating the arsenic disulfide with ammonia to reduce it to a powder, mixing it in the proportion of 1 to more than 5 of powdered calcium hydroxide in the cold, and adding enough water to make a paste.

It might be inferred that it is preferable to use arsenic sulfide for all kinds of skin where smoothness of grain is desired, but this is not necessarily so. All skins are not equally sensitive to injury through plumping. What may prove to be excessive plumping for goat skins may not have any deleterious effect at all on a calf skin and one type of calf skin might be more resistant to permanent distortion than



another. The greater speed of action and lower cost of sodium sulfide make its use preferable in all cases where it does no harm to the skins.

### Effect of Time in Sterile Limewater

Wilson and Daub<sup>34</sup> made a study, under the microscope, of the structure of calf skin during 7 months' contact with saturated limewater. They cut the butt of a fresh calf skin into pieces  $12 \times 36$  millimeters, soaked them in water at 7° C. for 1 day, and then put them all into a closed jar containing 1 liter of water and 27 grams of calcium hydroxide, an amount sufficient to keep the solution saturated at all times. The jar was kept in an incubator at 20° C. and pieces were drawn from it periodically. These were prepared for examination under the microscope by washing, dehydrating, imbedding, sectioning both vertically and horizontally, staining, mounting, and photographing, as described in Volume II. A second series was run in which the limewater was covered with a layer of toluene. Both series gave exactly the same results and both proved to be sterile, at least for 6 months. After 7 months the liquor without toluene showed a count of 1 per cc.; only a single colony developed from 1 cc. on agar during 4 days' incubation. We may thus infer that the changes occurring in the skin during the long contact with limewater were not caused by bacteria.

The first action noticeable was a slow disintegration of the cells of the Malpighian layer of the epidermis. By the end of 5 days this action had proceeded far enough to permit the easy removal of hair and epidermis by scraping with a blunt knife. Little further change was noticeable until after the third week.

For purposes of illustration, it was found best to use horizontal sections cut through the sebaceous glands. These were stained with Van Heurck's logwood and Daub's Bismarck brown. Plate 79 shows a section of the fresh skin after soaking in cold water for 1 day. In the center is a cross section of a hair running nearly at right angles to the plane of the page. Just below it, to left and right, is a pair of sebaceous glands, which furnish oil to the hair follicle. Surrounding both hair and glands are thin strands of elastin fibers. The dots are the stained nuclei of epithelial cells.

Plate 80 shows a similar cutting of the skin 3 weeks after being placed in the limewater. The glands have been attacked, and nearly all the epidermal cells surrounding the hair have disappeared, leaving it resting loosely in the empty follicle.

The elastin fibers still show up sharply after 3 weeks, but begin to break down rapidly about the fourth week. In Plate 81 the remnants of elastin fibers appear blurred. At the end of the fifth week the elastin fibers have entirely disappeared, as shown in Plate 82.

Many cell nuclei are still to be seen after 5 weeks, but are gradually destroyed and none are to be found after 3 months. The corneous layer of the epidermis and the hair appeared quite resistant to the action of lime for 15 weeks, but began to disintegrate slowly after this time, and had disappeared at the end of 7 months.

The collagen fibers, or leather-forming portion of the skin, remained sharply defined for 5 months, after which they assumed a blurred and glassy appearance and were slowly being hydrolyzed. After 7 months the skin had apparently lost its power to become fallen, or flaccid, when placed in neutral ammonium chloride solution. Pickling with sulfuric acid and salt caused it to shrink to a hard mass, resembling a dehydrated plate of gelatin. When tanned, it yielded a very thin and empty leather, indicating a very heavy loss of collagen during liming.

### Comparison of Acid and Alkaline Hydrolyses of Skin and Hair

In unhairing by liming, it is generally desired to hydrolyze the keratins sufficiently to loosen the hair and epidermis without causing any appreciable hydrolysis of the collagen. This makes it important to know the comparative rates at which keratin and collagen hydrolyze under different conditions. Merrill<sup>18</sup> carried out an interesting and important investigation to determine the rate of hydrolysis of skin and hair as a function of pH value, under definite conditions of time and temperature.

The skin used in the test was prepared by soaking 2 calf skins in several changes of pure water for 3 days, liming, unhairing and scudding, bating, and deliming with a solution of 0.01-normal hydrochloric acid containing 12 per cent sodium chloride. The entire skins were cut into pieces about 2 mm. square, brought to equilibrium with a borax solution of pH value = 7.7, and washed several days in cold, running tap water, followed by several changes of distilled water. The material was then dehydrated with absolute alcohol and the alcohol removed with xylene, which was allowed to evaporate from the pieces at room temperature. The resulting pieces of skin had the same appearance as the original skin, when soaked back with water. For the specimens of hair, brown hair was clipped from a number of skins, washed thor-

oughly in water, dried, and then freed from fatty matter with chloroform.

The general procedure was to soak 3-gram portions of skin or hair

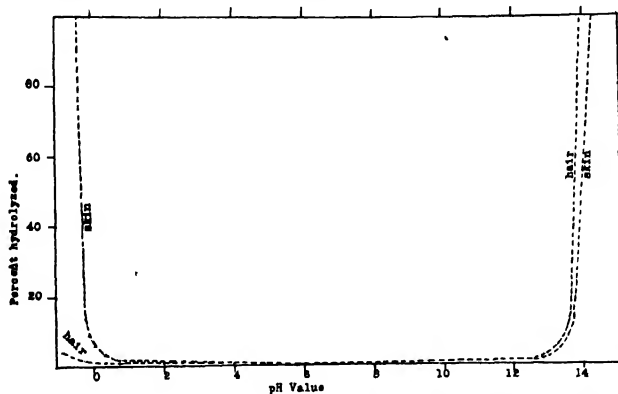


FIG. 45.—Effect of pH value on hydrolysis of skin and hair.  
Solutions: NaOH and HCl.  
Time: 24 hours.  
Temperature: 25° C.  
Independent variable: pH value.

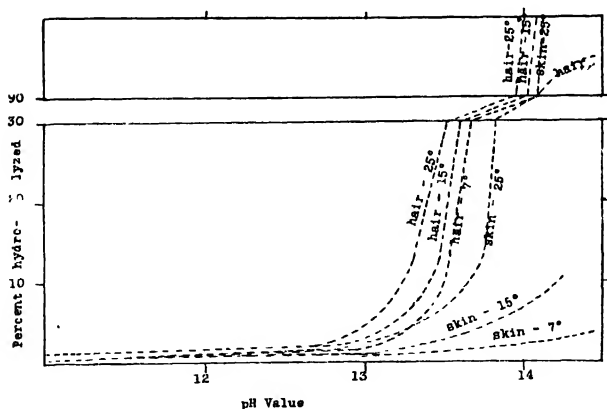


FIG. 46.—Effect of temperature on hydrolysis of skin and hair.  
Solutions: NaOH.  
Time: 24 hours.  
Temperature: 7°, 15°, and 25° C.  
Independent variable: pH value.

in 150 cc. of sodium hydroxide or hydrochloric acid solution of known hydrogen-ion concentration for a definite time at fixed temperature. The solutions were protected from bacterial action by covering them with a layer of toluene. At the end of the digestion period the solutions

were filtered and the dissolved nitrogen was determined in an aliquot of the filtrate and assumed to be a measure of the extent of the hydrolysis. No buffers were used and the per cent of the protein

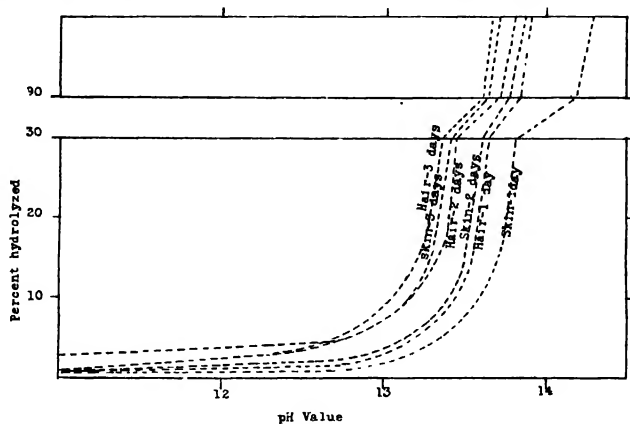


FIG. 47.—Effect of time of digestion on hydrolysis of skin and hair.

Solutions: NaOH.

Temperature: 25° C.

Time: 1, 2, and 3 days.

Independent variable: pH value.

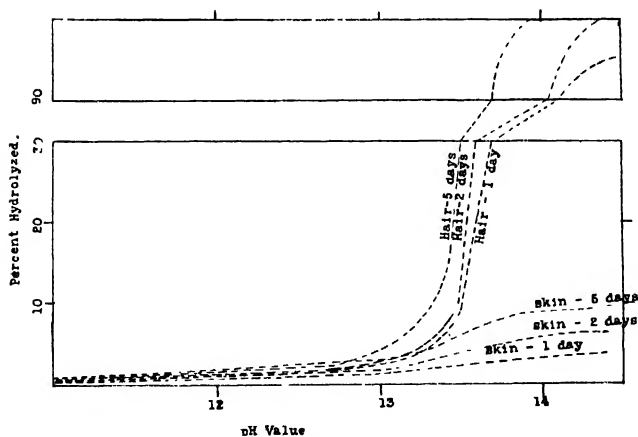


FIG. 48.—Effect of time of digestion on hydrolysis of skin and hair.

Solutions: NaOH.

Temperature: 7° C.

Time: 1, 2, and 5 days.

Independent variable: pH value.

hydrolyzed was plotted against the final pH value of the solution, measured electrometrically. The results are shown in Figs. 45, 46, 47, 48, and 49.

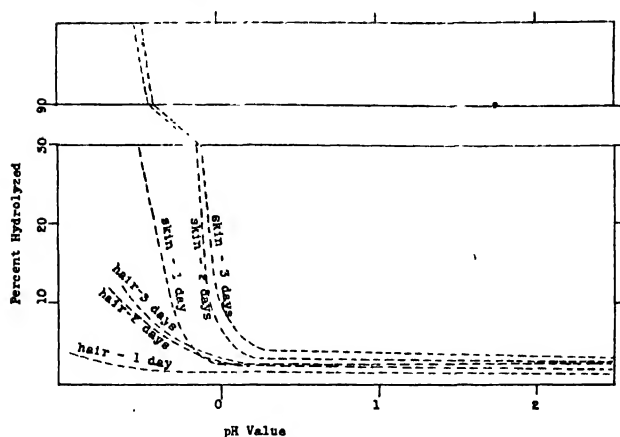


FIG. 49.—Effect of time of digestion on the hydrolysis of skin and hair.

Solutions: HCl.

Temperature: 25° C.

Time: 1, 2, and 3 days.

Independent variable: pH value.

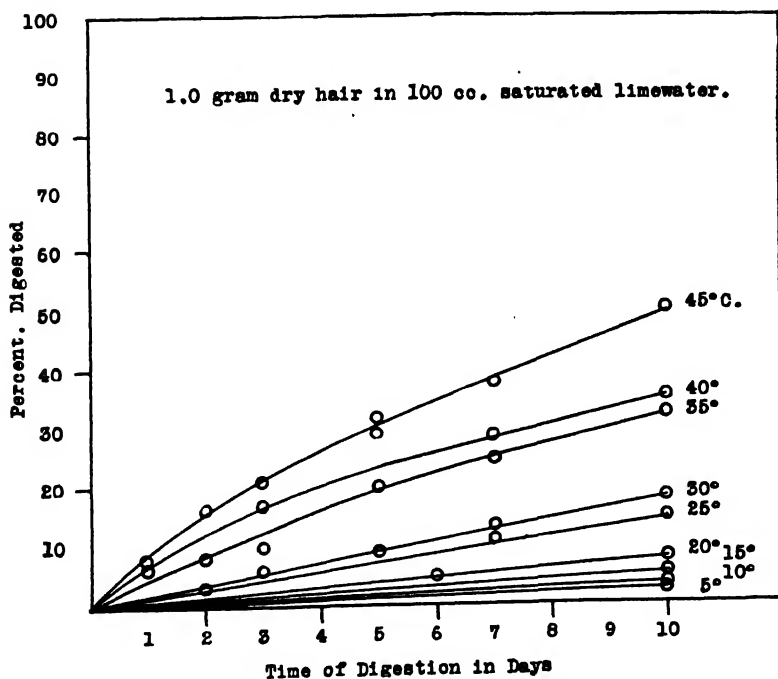


FIG. 50.—Hydrolysis of hair by saturated limewater as a function of time and temperature.

Fig. 45 shows the per cent of total nitrogen hydrolyzed in 24 hours at 25° C. at pH values over the entire range from 4-molar hydrochloric acid to 4-molar sodium hydroxide solution. In acid solution skin is more easily hydrolyzed than hair at the same pH value, while in alkaline solution hair is more easily hydrolyzed than skin. Between the pH values 1 and 12, relatively little hydrolysis takes place, but as the pH values are extended beyond this range, there is a very abrupt increase in hydrolysis, rapidly running to completion.

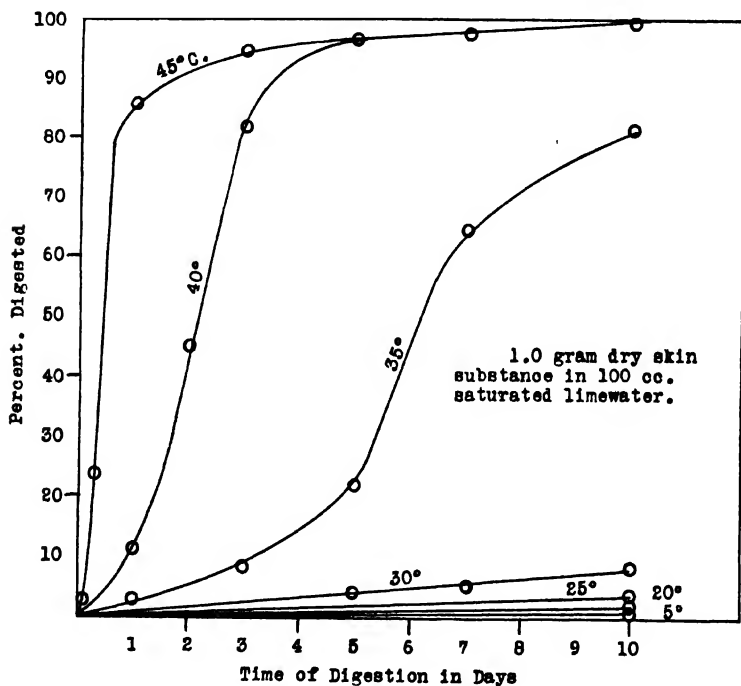


FIG. 51.—Hydrolysis of skin by saturated limewater as a function of time and temperature.

Fig. 46 shows the effect of temperature, from 7° to 25°, in alkaline solutions. With increasing temperature the pH value at which complete hydrolysis occurs shifts to lower pH values, the effect being more pronounced with skin than with hair. The effects of temperature and of time of digestion are given in Figs. 47 and 48 for alkaline solutions. The effect of time for acid solutions is shown in Fig. 49. The effect of increasing the time of digestion is similar to that of increasing the temperature.

Merrill and Fleming<sup>22</sup> measured the rate of hydrolysis of skin and

hair in saturated limewater as a function of time and temperature, covering the range from 5° to 45° C. Their very interesting results are shown in Figs. 50, 51, and 52. The calf skin and hair used in these tests were purified and dried as indicated above in Merrill's experiments on the acid and alkaline hydrolysis of skin and hair. One-gram portions of dry skin or hair were digested with 100 cc. portions of lime-

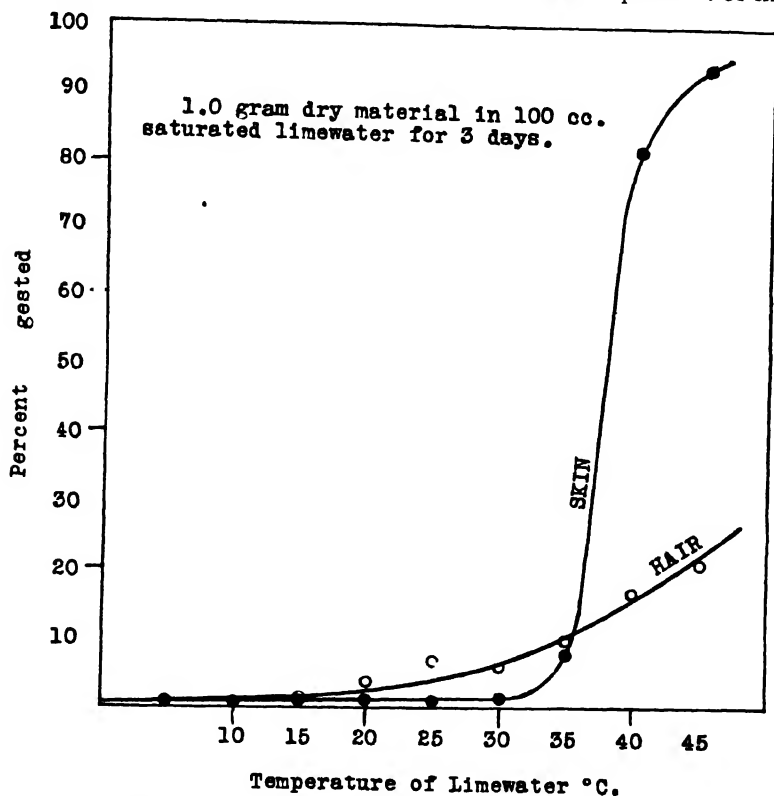


Fig. 52.—Effect of temperature upon the hydrolysis of skin and hair by saturated limewater.

water containing excess of undissolved lime for the period of time and at the temperature indicated on the charts. At the end of the digestion period, each liquor was filtered and nitrogen determined in an aliquot of filtrate. The per cent of protein digested was calculated from the ratio of dissolved nitrogen to total nitrogen.

With rise of temperature, the digestion of both skin and hair is increased. Up to 30° C., hair is digested much more rapidly than skin, but at about 35° there is a very marked increase in hydrolysis

of skin. At 40° the rate of solution of skin is enormously greater than that of hair. This is shown very clearly in Fig. 52.

In liming, it is generally desirable to get the greatest rate of digestion of keratin with least digestion of collagen. Evidently this is best accomplished by liming at temperatures not higher than 30°.

### Effect of Sulfides

The addition of sodium sulfide, or other sulfides, to a lime liquor greatly increases the unhairing action. Tanners have long known this and used sulfides as sharpening agents in liming, but it is only very recently that any plausible explanations have been given of the chemical reactions involved.

The effect upon the epidermal system of adding sulfide to a lime liquor depends upon the amount used. In concentrations of about 0.01-molar sulfhydrate, the effect is primarily to decrease the time required to loosen the hair. In much higher concentrations, the hair is destroyed, being reduced to a pulp which can be washed off from the skin. Some authors distinguish rather sharply between the hair-loosening and hair-pulping actions, but it is probable that the actions are the same, differing only in degree. In the hair-loosening action, it is the newly formed epithelial cells that are destroyed. These cells ultimately would have become the hardened tissues of the corneous layer of the epidermis or of the hair shafts, had the animal continued to live. The older and drier keratins are more resistant to attack than the young keratin, but there is no reason to believe that the action of sulfide on the two kinds is not fundamentally the same.

In studying the effect of sulfides on the alkaline hydrolysis of skin and hair, Merrill<sup>19</sup> used essentially the same method that was employed in the study of acid and alkaline hydrolysis of skin and hair just described. A definite quantity of purified skin or hair was treated with the sulfide liquor being studied at 25° C. and the degree of hydrolysis was measured as a function of concentration of sulfide, pH value, and time. The per cent of total nitrogen passing into solution was taken as the measure of the hydrolysis. The amount of sulfide absorbed by the hair or skin was determined by analysis of the liquor before and after the digestion, using the volumetric zinc precipitation method with nitroprusside indicator.

The solutions employed were (1)  $\text{NaOH} + \text{NaSH}$ ; (2)  $\text{Ca}(\text{OH})_2 + \text{NaSH}$ ; and (3)  $\text{Ca}(\text{OH})_2 + \text{Ca}(\text{SH})_2$ .  $\text{NaSH}$  was prepared by passing pure  $\text{H}_2\text{S}$  into 2-molar  $\text{NaOH}$  solution until the solution contained



$\text{Na}^+$  and  $\text{SH}'$  in equivalent amounts, as shown by analysis.  $\text{Ca}(\text{SH})_2$  was prepared in a similar manner by passing  $\text{H}_2\text{S}$  into a suspension of  $\text{Ca}(\text{OH})_2$ .

In comparing the action of sulfides on skin and hair, series were run in which increasing amounts of  $\text{NaSH}$  or  $\text{Ca}(\text{SH})_2$  were added to saturated  $\text{Ca}(\text{OH})_2$  containing an excess of undissolved lime. The digestion period was one day and the temperature  $25^\circ \text{C}$ . The results are shown in Fig. 53.

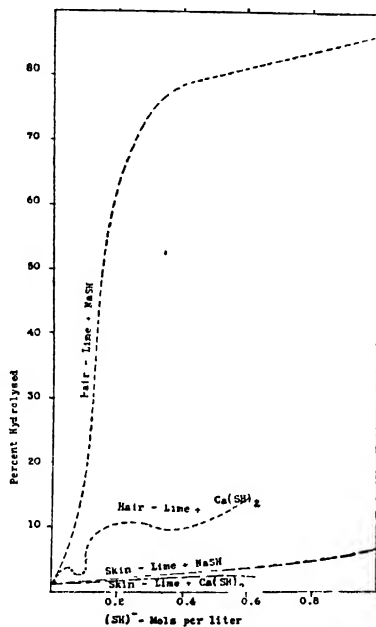


FIG. 53.—Hydrolysis of skin and hair as a function of sulfide concentration.

Time, 1 day.

Temperature,  $25^\circ \text{C}$ .

In the digestion of skin, the sulfide concentration of the liquor was the same after the digestion as before, indicating no absorption, but in the digestion of the hair there was a drop in concentration of sulfide in every case. In the solution of 0.2-normal  $\text{NaSH}$  the drop was from 0.200 to 0.134 and in the solution of 0.2-normal  $\text{Ca}(\text{SH})_2$  it was from 0.200 to 0.156. At this concentration, there was no measurable absorption of sulfhydrylate by skin in either case and the per cent hydrolyzed was less than two; 44 per cent of the hair was hydrolyzed by 0.2-normal  $\text{NaSH}$  and only 8 per cent by 0.2-normal  $\text{Ca}(\text{SH})_2$ . In a normal solution of  $\text{NaSH}$ , 85 per cent of the hair was hydrolyzed. This experiment

shows that the hydrolysis of hair is connected with the absorption of sulfide from solution.

Measurement of the effect of concentration of sulphhydrate in a lime liquor presented a difficult problem in that variation in concentration of sulphhydrate causes a variation in solubility of the lime and, consequently, a variation in pH value. Moreover, the magnitude of the change in pH value produced by the addition of sulfide to a lime liquor cannot be accurately measured in the presence of large amounts of sulfide, owing to the poisoning action of the sulfide on the hydrogen electrode and the color reactions which sulfides seem to undergo with all

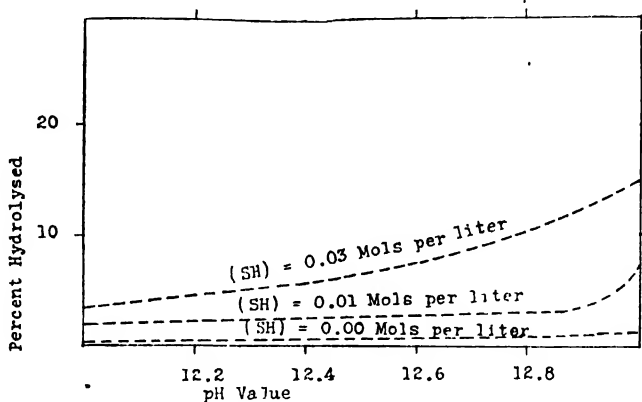


FIG. 54.—Hydrolysis of hair as a function of pH value at different initial concentrations of sulfide

Solutions, NaOH + NaSH

Time, 1 day

Temperature, 25° C.

the indicators suitable for the pH range in question. This difficulty was overcome by the following procedure: A series of solutions was prepared, all containing the same amount of NaSH, and containing increasing amounts of NaOH. The amount of sulfide was made small in order that the effect on the pH value might be as small as possible. The pH values of these solutions were calculated from the concentrations of NaOH and NaSH, and the hydrolysis constant of NaSH. The results obtained with such a series gave the effect of increasing OH concentration on the hydrolysis of hair, at a constant SH concentration. Other series were run at different concentrations of sulfide. The results of these series, when per cent of hydrolysis was plotted against pH value, gave a series of curves, and the vertical distance between any two curves gave the difference in per cent of hydrolysis caused by a

difference in sulfide concentration, at a constant pH value. The results of these experiments are given in Fig. 54.

At any given sulfide concentration, the rate of hydrolysis of hair increases with increasing pH value, while, at any given pH value, the rate of hydrolysis increases with sulfide concentration. This throws more light on the curves in Fig. 53. With increasing concentration of NaSH both the sulfide concentration and pH value are increased, resulting in a very rapid increase in per cent hydrolysis. With increasing concentration of  $\text{Ca}(\text{SH})_2$ , the solubility of the lime is decreased and a lowered pH value results; two factors, having an opposite effect on the hydrolysis of hair, are being varied simultaneously, and the

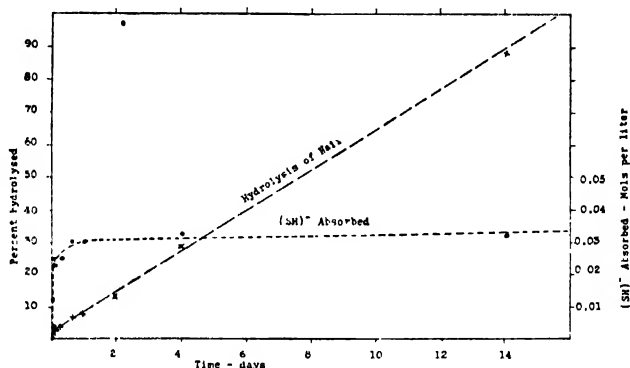


FIG. 55.—Hydrolysis of hair and absorption of sulfide as a function of time.

Solutions,  $\text{Ca}(\text{SH})_2 + \text{Ca}(\text{OH})_2$ .  
Temperature,  $25^\circ \text{C}$ .

result is the irregular curve shown in the figure. It was demonstrated by duplicate test that the maxima are not the result of experimental error.

Fig. 55 shows the hydrolysis of hair and absorption of sulfide from a 0.136-normal solution of  $\text{Ca}(\text{SH})_2$  saturated with lime at  $25^\circ \text{C}$ . as a function of time. Three grams of hair were treated with 150 cc. of the liquor. The hydrolysis of hair is practically a straight-line function of time, but nearly all of the absorption of sulfide takes place in the first half hour, indicating that the solution of hair is not determined by sulfide absorption alone.

Merrill's work brings out the following important facts:

- 1—Sulfides increase the hydrolysis of hair at a given pH value, but have no appreciable hydrolytic action on skin.
- 2—Sulfide is absorbed by hair but not by skin.

3—No definite relation has been found between the amount of sulfide absorbed and the amount of hair hydrolyzed.

4—In the absorption of sulfide, equilibrium is established in a few hours. The hydrolysis of hair, on the other hand, continues at a nearly uniform rate for days.

5—In the presence of sulfide, the effect upon the hydrolysis produced by increasing the pH value of the solution is very much greater than in the absence of sulfide.

These facts lend themselves to the following interpretation:

1—There is a reaction between keratin and the SH ion.

2—This reaction so alters the structure of the protein that the residues are more readily attacked by the OH ion.

Stiasny<sup>29</sup> long ago showed that the unhairing of skins was dependent upon both the sulfide and the hydroxide concentrations. He found that the best results were obtained in the presence of equivalent amounts of SH and OH, and that the increasing SH beyond this ratio had an inhibiting influence on the unhairing. This is true over a small range of sulfide concentration, as is shown in one of the curves of Fig. 53. The effect, however, is due to the diminution of the pH value. If the concentration of OH ions is maintained constant, the effect of increasing sulfide concentration is to increase the amount of hair dissolved, no matter what the ratio of OH to SH may be.

Merrill presented the following hypothesis: the action of alkaline sulfide solutions on hair proceeds in two steps, the first being a rapid reaction between keratin and sulfide and the second the hydrolysis of the altered keratin by the hydroxide. This hypothesis enabled him to make a prediction that could be tested by experiment. If this hypothesis is valid, after the reaction between hair and sulfide has occurred the further hydrolysis should go on just as well in a solution free from sulfide as in the solution in which the initial sulfide-keratin reaction took place.

This prediction was tested by the following experiment: Two samples of hair were treated with the same solution of  $\text{Ca}(\text{OH})_2$  and  $\text{Ca}(\text{SH})_2$ . Sample A was digested for 24 hours. In the case of Sample B, however, four-fifths of the sulfide solution was replaced by lime-water after one hour. The digestion was then continued for the 24-hour period. Nitrogen determinations were made on the removed liquor from B, and on the final solutions obtained from A and B. Sulfide determinations were made on the same solutions. A third

sample, *C*, was digested with a lime-sulfide solution made so as to have the same final sulfide concentration as the solution in contact with *B*, after the replacement of the initial solution with lime water.

The nitrogen dissolved in the period between the end of the first hour and the end of the twenty-fourth hour was calculated. The results are given in Table XXI.

TABLE XXI

EFFECT OF CONCENTRATION OF SULFIDE ON HYDROLYSIS OF HAIR AFTER INITIAL DIGESTION

(Solutions,  $\text{Ca}(\text{OH})_2 + \text{Ca}(\text{SH})_2$ ; temperature,  $25^\circ \text{C}$ .)

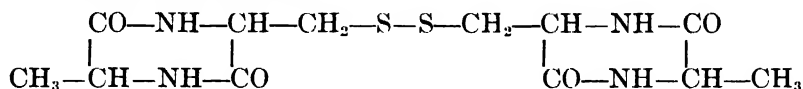
Sample	Mols (SH)- Per Liter			Grams Nitrogen Digested In Period		
	Start	1 Hour	24 Hours	1 Hour	1 to 24 Hours	24 Hours
<i>A</i>	0.119	0.098	0.098	0.0131	0.0971	0.1102
<i>B</i>	0.119	0.098	0.019	0.0131	0.0801	0.0932
		0.019 <sup>a</sup>				
<i>C</i>	0.026	0.018	0.018	0.0084	0.0013	0.0097

<sup>a</sup> 120 cc. of liquor replaced by  $\text{Ca}(\text{OH})_2$  solution after 1 hour.

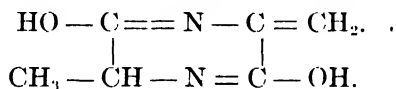
In general, the results of this experiment are in accordance with the theory. This is certainly true in a qualitative sense. The amount of nitrogen dissolved between the end of the first and twenty-fourth hours is very nearly as great in the case of Sample *B*, where the bulk of the sulfide solution was removed after one hour, as in Sample *A*, where the entire quantity of sulfide was left in contact with the sample for the full digestion period. On the other hand, the nitrogen digested in this period in Sample *B* is sixty times as great as in a solution of the same sulfide concentration, Sample *C*, in contact with hair which had not been previously treated with stronger sulfide.

A number of investigations have been carried out recently to throw some light on the nature of the reaction between sulfide and the keratin molecule. Kaye and Marriott<sup>12</sup> observed that hair freed from sulfur by the action of alkali is no longer capable of combining with sulfide and conclude that the point of attack by the sulfide is the cystine group of the keratin molecule. Bergmann and Stather<sup>4</sup> found that hair hydrolyzed by sodium sulfide yields much less cystine than hair hydrolyzed by acid, indicating that the cystine group is attacked during the reaction with sulfide. In later works they<sup>5</sup> suggested that the hydrolysis might take place either by the separation of the primary aggregates loosely linked together to form the keratin molecule or by the destruction of the aggregates themselves, the latter seeming the more likely because of the disappearance of cystine during the alkaline hydrolysis of keratin. Cystine, dialanyl cystine, and dileucyl cystine

are attacked by alkali, although slowly, but the anhydrides dialanylecystine-dianhydride and dileucyl-cystine-dianhydride are very rapidly attacked by alkali, the sulfur being removed along with hydrogen, forming sulfides and polysulfides, and the remainder of the molecule forming piperazine derivatives. Stather<sup>28</sup> showed that the compound dianalyecystine-dianhydride:



is readily split by dilute alkali at the —S—S— bond, yielding sulfides, polysulfides, and 3-methylene-6-methyl-2, 5-dioxypiperazine:



A similar type of reaction occurs also with the corresponding dileucyl compound. The behavior of these cystine-containing compounds is considered analogous to that of keratin.

Atkin and Thompson<sup>2</sup> suggest that cystine is reduced to cysteine by the action of sulfide. The cysteine then acts as an oxygen carrier, being alternately oxidized by the air and reduced by the remainder of the molecule, which is thus ultimately destroyed by oxidation. The suggestion that the first action is one of reduction of the cystine is confirmed by Merrill's<sup>21</sup> work on stannous chloride as an unhairing agent, which will be described presently.

### Action of Ammonia on Collagen

Used lime liquors contain ammonia resulting from the decomposition of protein matter. The belief has been very widespread that ammonia hydrolyzes collagen readily so that its presence in considerable quantities in a lime liquor will result in considerable losses in hide substance. But this view has recently been shown to be false by Merrill,<sup>20</sup> who found that saturated limewater containing ammonia exerts no greater hydrolytic action on collagen than limewater free from ammonia.

Merrill's method was the same in principle as that used to measure the hydrolysis of skin and hair, described above. He used purified calf skin cut into squares of 2 mm. side and Standard hide powder previously freed from fat and water soluble material. Two-gram samples of calf skin or hide powder were placed in stoppered bottles with 200 cc. of the solution containing ammonia. A little toluene was

added to check bacterial action. The bottles were kept in constant temperature baths for definite periods of time, and the skin or hide powder was filtered off and washed thoroughly to remove the ammoniacal solution. Undissolved collagen was determined by carrying out a Kjeldahl determination on the entire insoluble residue and dissolved collagen was estimated by difference. Blanks containing no ammonia were run in every case.

In Fig. 56 are given the results of 3 series of experiments in each of which the independent variable was the concentration of ammonia.

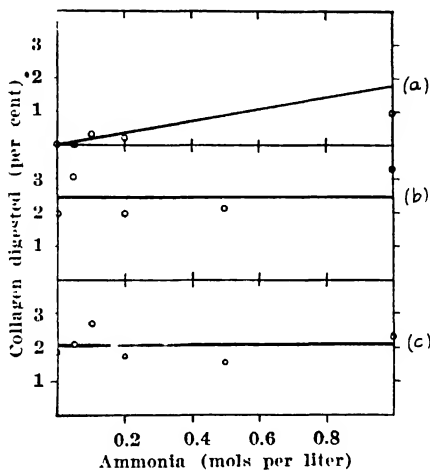


FIG. 56.—Action of ammonia on calfskin. (Time, 3 days; temperature, 25° C.)  
 (a) Ammonia in distilled water. (b) ammonia in saturated calcium hydroxide;  
 (c) ammonia in 0.05 N sodium hydroxide.

The first series was made with water and ammonia up to molar concentration. The per cent of collagen dissolved from calf skin increases with the concentration of ammonia, which is to be expected, since ammonia is a weak base and the pH value increases with concentration. Moeller<sup>23</sup> made a similar finding with hide powder. In the second and third series, where ammonia was added to saturated limewater and 0.05-normal sodium hydroxide, respectively, the concentration of ammonia was without effect upon the hydrolysis, presumably because it had no appreciable effect upon the pH value. This shows that ammonia has no specific action upon collagen. Exactly the same effect was observed with hide powder, which is much more readily hydrolyzed than the cubes of calf skin.

As it was thought possible that small quantities of ammonia in limewater might have some action on collagen after long contact, a series was run in which all the members contained ammonia in 0.1-molar concentration, the independent variable being time. A corresponding series was run with limewater containing no ammonia. The two series gave the same results for all periods of digestion as shown

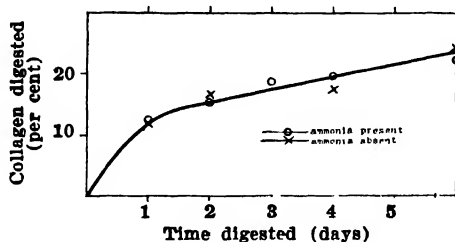


FIG. 57.—Effect of time on action of ammonia (in saturated calcium hydroxide) on hide powder. (Temperature, 25° C.)

in Fig. 57. This indicates that ammonia in lime liquors is without action on collagen up to 6 days of contact.

The possibility remained that ammonia, or the ammonium ion, might exert a specific hydrolyzing action on collagen at some pH lower than those hitherto employed. This is of importance in connection with possible damage to skins by ammonia during bating. Two series

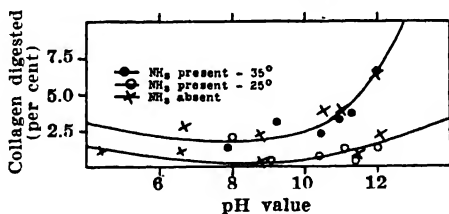


FIG. 58.—Action of ammonia (in buffered solutions) on calfskin at different pH Values. (Time, 1 day.)

were run at pH values covering the range from 7 to 12, in one of which all the members contained ammonia while the other served for blanks. The experiment was run both at 25° and at 35° C., the latter temperature being somewhat above that commonly used in bating. The results given in Fig. 58 show that at no pH value in the range covered does ammonia have any appreciable action on skin.

As a drastic test of the conclusion previously drawn, that ammonia is without appreciable action on calfskin, three strips of fresh skin were placed in solutions containing, respectively, 0.5 N, 8 N, and con-



concentrated ammonia, and allowed to stand for six weeks. Upon removal from the ammonia, the strips were greatly swollen, but otherwise unchanged except for the loss of hair. The pieces were pickled with acid and salt, and tanned with vegetable tanning materials. After tanning, they appeared to be good leather, showing no looseness of grain, no emptiness, and no weakness on tearing. So far as can be told from a small strip, the skin had suffered no appreciable damage even from immersion in concentrated aqua ammonia for six weeks.

It is safe to conclude that ammonia has no appreciable action on the collagen fibers that make up the bulk of the skin and that ammonia is not responsible for the large losses of hide substance and low leather yields for which it has occasionally been blamed. One must look to other causes for an explanation of such losses, when they occur.

#### Ammonia as an Unhairing Agent.

Ammonia in pure aqueous solution is a powerful unhairing agent, but how much this property is effective in liming depends upon conditions, a study of which was recently made by Merrill.<sup>20</sup> If a skin is placed in a 2-molar solution of ammonia, the hair will slip easily after a few hours and the unhaired skin will be but little swollen. The difficulty of working with strong ammonia solutions has made this method impractical. Old lime liquors contain considerable amounts of ammonia, which may play a part in the unhairing action, under certain conditions.

The only previous critical work done on this subject was that of Stiasny,<sup>20</sup> who reached the following conclusions:

(1) The unhairing is a specific property of ammonia, as it is manifested at pH values around 11, where the unhairing action of hydroxyl ion is negligible.

(2) The unhairing is inhibited by divalent cations. When ammonium salts are added to saturated lime solutions, there is no improvement in the unhairing action of the lime. When calcium salts, or salts of barium or zinc, are added to ammonia, its unhairing power is lost. From this it follows that the presence of ammonia in old lime liquors has no influence on the unhairing. The explanation suggested for the inhibition of ammonia unhairing by calcium was the formation of complex ions, of the type  $\text{Ca}(\text{NH}_3)_6$ , such as ammonia is known to form with divalent cations.

If this explanation is true, it follows that a given amount of

calcium ion can inactivate, as it were, only a limited amount of ammonia, and that if this ratio of ammonia to calcium is exceeded, the excess ammonia should manifest its unhairing powers despite the presence of lime. Merrill's experiments were arranged so as to cover this point. A series of solutions was made up, each member of which contained 1.0 gram of lime in 200 cc., and in which the different members contained increasing amounts of ammonia. A parallel series was run with the same quantities of ammonia, but no lime. Strips from the head of a fresh calfskin, each about  $1 \times 4$  inches, were placed in the solutions, and examined for unhairing action at hourly intervals.

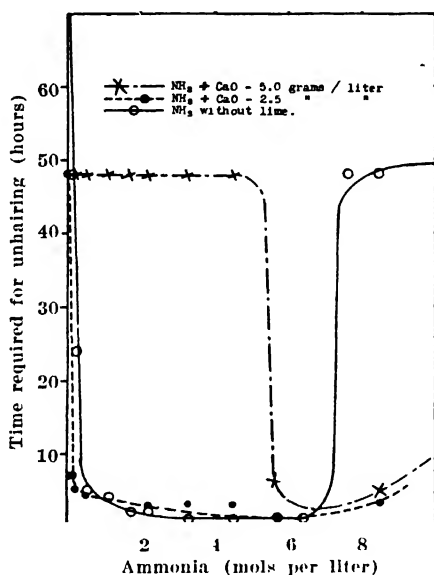


FIG. 59.—Rate of unhairing with lime and ammonia.

Unhairing was considered satisfactory when the hair slipped easily under gentle pressure from a blunt knife.

The results of these experiments are given in Fig. 59, in which the time required for satisfactory unhairing is plotted as a function of ammonia concentration. In the absence of lime, unhairing takes place very quickly, even in fairly dilute solutions of ammonia. When the concentration rises above a certain point, the time required for unhairing becomes much longer. In the presence of lime, the ammonia produced no unhairing until the concentration had risen to between 5 and 6 *N*. Above this concentration, rapid unhairing took place, comparable to that obtained with ammonia alone at lower concentrations.

The results thus far obtained were in accordance with the complex ion theory of the inhibition of ammonia unhairing by lime. A closer examination of the data, however, reveals the surprising fact that if the lime prevents the ammonia from acting on the skin by combining with it, then the formula of the complex ion must be about  $\text{Ca}(\text{NH}_3)_{60}$ , for in the experiments just described 1.0 gram, or about 0.02 mol, of lime was capable of inhibiting the action of 200 cc. of 5 *N* ammonia, containing 1 mol of ammonia.

On the basis of complex formation, different quantities of lime should inhibit proportional amounts of ammonia. To see whether this is the case, the experiments were repeated, using one-half the original quantity of lime (0.5 gram in 200 cc.) with the same concentrations of ammonia. The results of these tests are given in Fig. 59. At this lower concentration, the lime has no inhibiting action; in fact, at some concentrations of ammonia, the lime seems to aid unhairing.

These experiments seemed to make it very doubtful that calcium ion alone has anything to do with the inactivation of the ammonia. To settle this point, the influence of a neutral calcium salt (calcium chloride) on the unhairing was investigated. A series in which all the members contained 2 *N* ammonia and increasing quantities of calcium chloride up to 2 *N* was set up. So far as could be detected, all the strips unhaird with equal ease in the same length of time, indicating that neutral calcium salts do not prevent unhairing by means of ammonia.

Having thus proved that the calcium ion is not solely responsible for preventing unhairing by ammonia in the presence of lime, the next step was to investigate the effect of hydroxyl-ion concentration. To determine the influence of hydroxide ion upon ammonia unhairing, strips of calf skin were treated with a molar solution of ammonia containing increasing quantities of barium hydroxide, which was chosen instead of calcium hydroxide because of its greater solubility. A similar series was run with ammonia and sodium hydroxide, and blanks were run with both sodium hydroxide and barium hydroxide without the ammonia. The time required for unhairing each strip was determined as before, and the pH value of each solution was determined electrometrically.

The results obtained with ammonia and barium hydroxide are given in Fig. 60. As the concentration of barium hydroxide, and, in consequence, the pH value, increases, the rate of unhairing increases until an alkalinity of pH 12 is reached. Further increase in pH value causes the unhairing to be greatly retarded. The effect obtained with the

two different concentrations of lime (Fig. 59) is thus explained, as with the smaller quantity of lime saturation was not maintained, and the pH value probably dropped below 12.

With sodium hydroxide a similar minimum in the time required for unhairing was noted, but the retarding effect at pH values above 12 was much less pronounced.

The retardation of ammonia unhairing by increase of pH value above 12 seems to be related to the swelling of the skin and the fol-

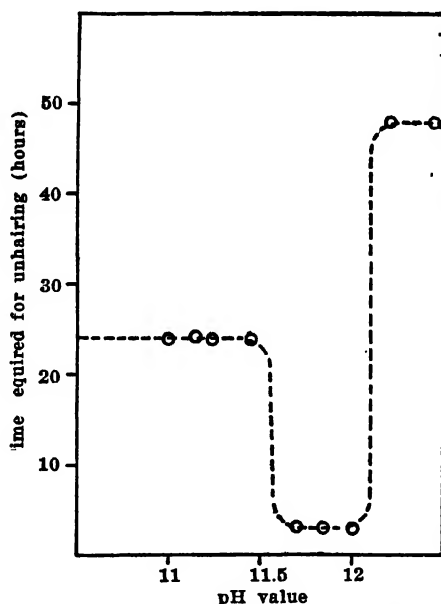


Fig. 60.—Effect of pH value on rate of unhairing with ammonia in barium hydroxide solution.

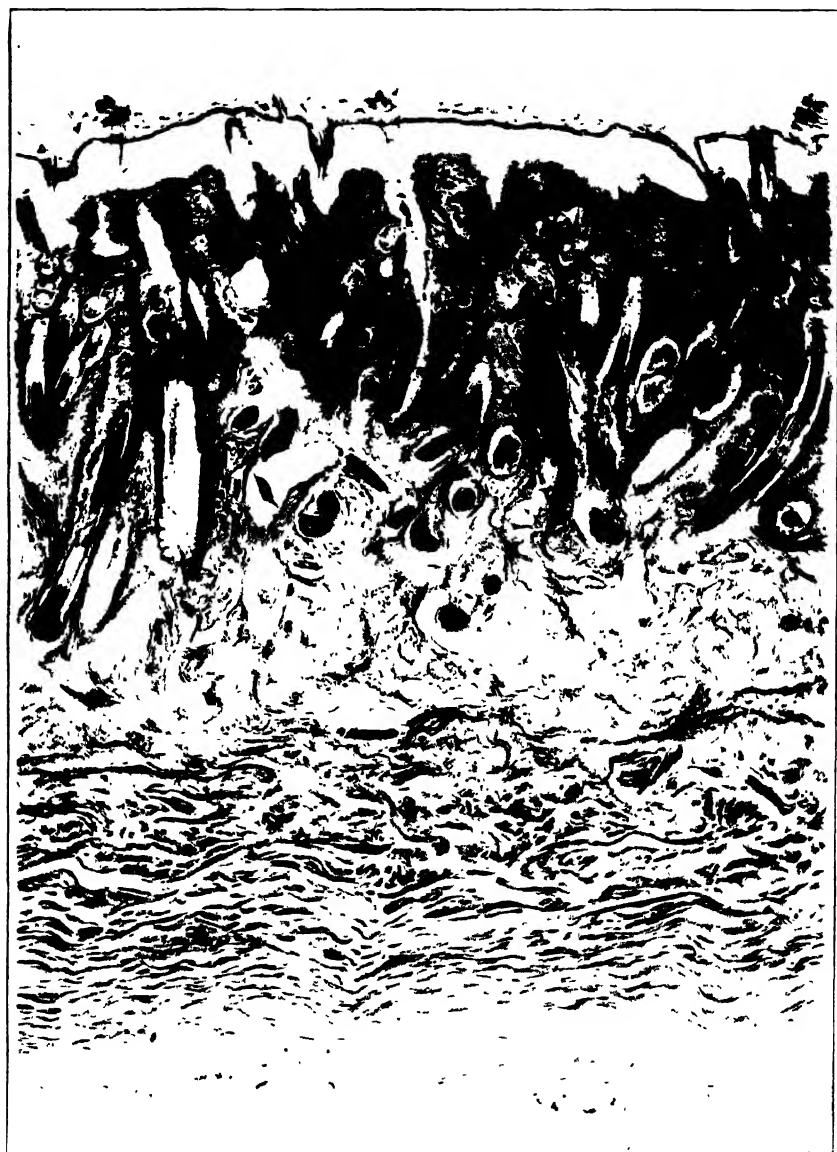
lowing explanation has been offered. The bulb of the hair in the follicle is of larger diameter than the shaft. The ammonia acts upon the tender epithelial cells lining the hair follicle, thus separating the hair from the lining of the follicle and permitting its easy removal when the skin is in a flaccid condition. But when the skin swells, the opening of the follicle becomes constricted and the hair, because of its large bulb, is held tightly again. In many cases it was noticed that the hairs were loosened after a short exposure to ammonia, but that they became tight again after longer treatment. After this swelling has occurred, unhairing cannot take place without a more drastic action on the hair bulb or the cells immediately surrounding it, making



Plate 73.—Hauling Skins from Lime Liquor.



Plate 74.—Scudding Calf Skins on the Beam



**Plate 75.—Vertical Section of Sheep Skin.**

(After 12 hours in sweat chamber.)

Location, butt

Thickness of section: 20  $\mu$ .

Stains Van Heurck's logwood,

Daub's bismarck brown.

Eye-piece, none.

Objective: 16-mm

Wratten filter H-blue grc

Magnification: 45 diameters



**Plate 76.—Vertical Section of Thermostat Layer of Sheep Skin.**

(After 42 hours in sweat chamber )

Location: butt.

Thickness of section: 20  $\mu$ .

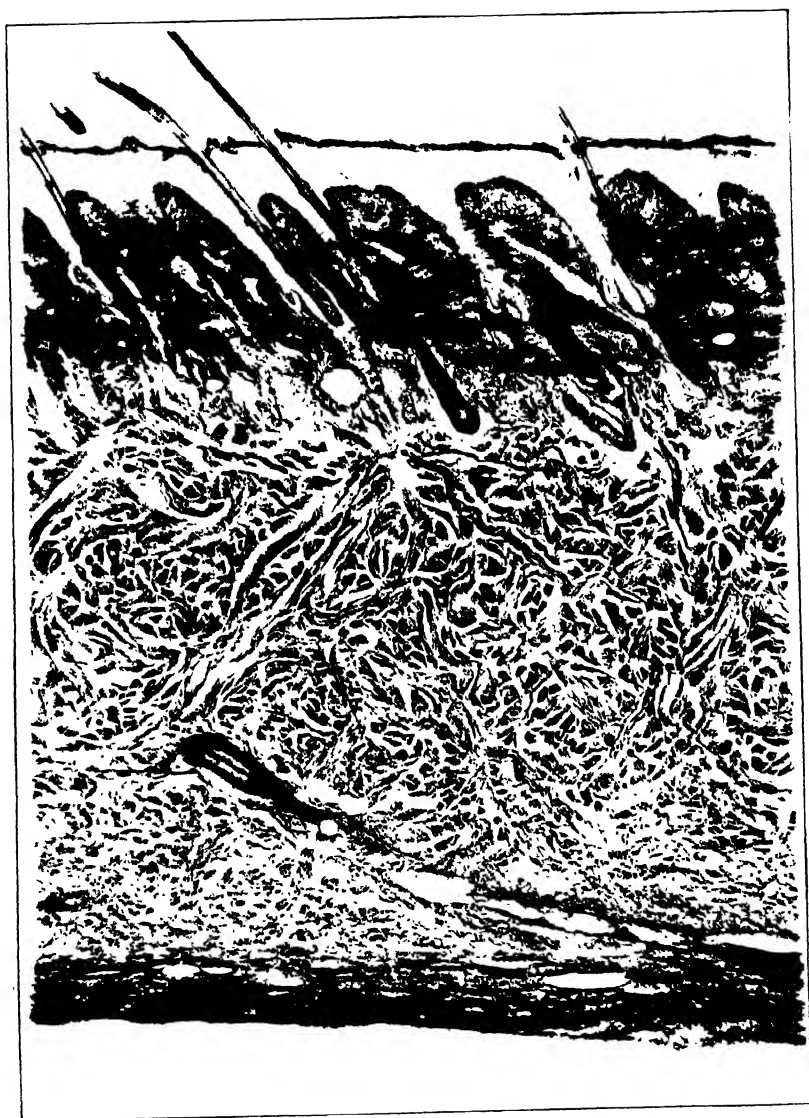
Stains. Van Heurck's logwood,  
Daub's bismarck brown.

Eye-piece. 5X.

Objective: 16-mm.

Wratten filter: H-blue green.

Magnification: 135 diameters.



**Plate 77.—Vertical Section of Calf Skin.**  
(After 48 hours in lime liquor )

Location: butt.  
Thickness of section: 40  $\mu$ .  
Stains: Weigert's resorcin-fuchsin  
and picro-red.

Eyepiece, none  
Objective: 32-mm.  
Wratten filters: B-green; E-orange.  
Magnification, 25 diameters.





**Plate 78.—Vertical Section of Thermostat Layer of Calf Skin.**  
(After 48 hours in lime liquor)

Location, butt.  
Thickness of section: 40  $\mu$ .  
Stains: Weigert's resorcin-fuchsin  
and picro-red.

Eyepiece: 5X.  
Objective: 16-mm.  
Wratten filters: B-green; E-orange.  
Magnification: 135 diameters.



**Plate 79.—Horizontal Section of Calf Skin through Sebaceous Glands.**  
(Before lining)

Location: butt.

Thickness of section: 30  $\mu$ .

Stains: Van Heurek's logwood,

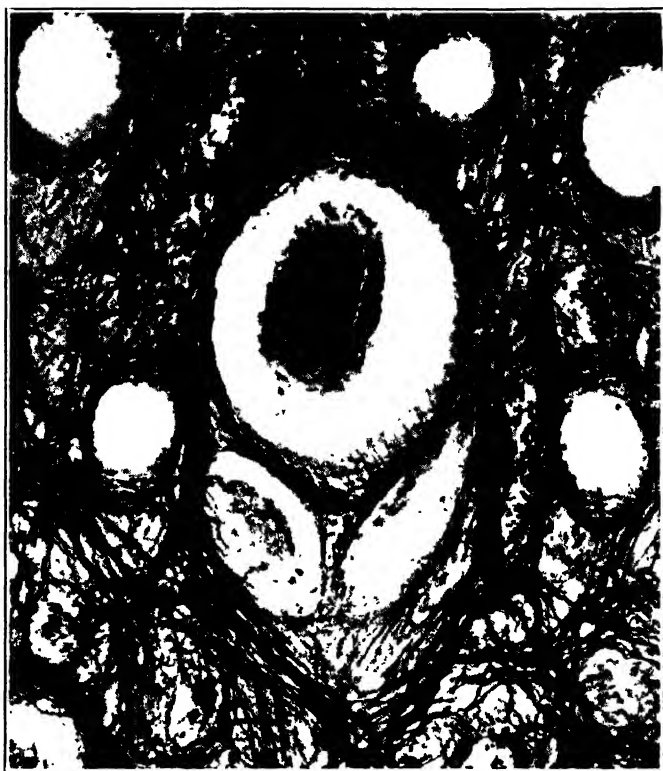
Daub's bismarck brown.

Eye-piece: 5X

Objective: 8-mm

Wratten filter: H-blue green.

Magnification: 190 diameters.



**Plate 80.—Horizontal Section of Calf Skin through Sebaceous Glands.**  
 (After 3 weeks' contact with sterile lime-water.)

Location: butt.

Thickness of section: 30  $\mu$ .

Stains: Van Heurck's logwood

Daub's bismarck brown.

Eye-piece: 5X.

Objective: 8-mm

Wratten filter: H-blue green.

Magnification 190 diameters.



**Plate 81.—Horizontal Section of Calf Skin through Sebaceous Glands.**  
 (After 4 weeks' contact with sterile lime water )

Location: butt.

Thickness of section: 30  $\mu$

Stains: Van Heurck's logwood.

Daub's bismarck brown

Eye-piece: 5X.

Objective: 8-mm

Wratten filter, H-blue green

Magnification: 190 diameters

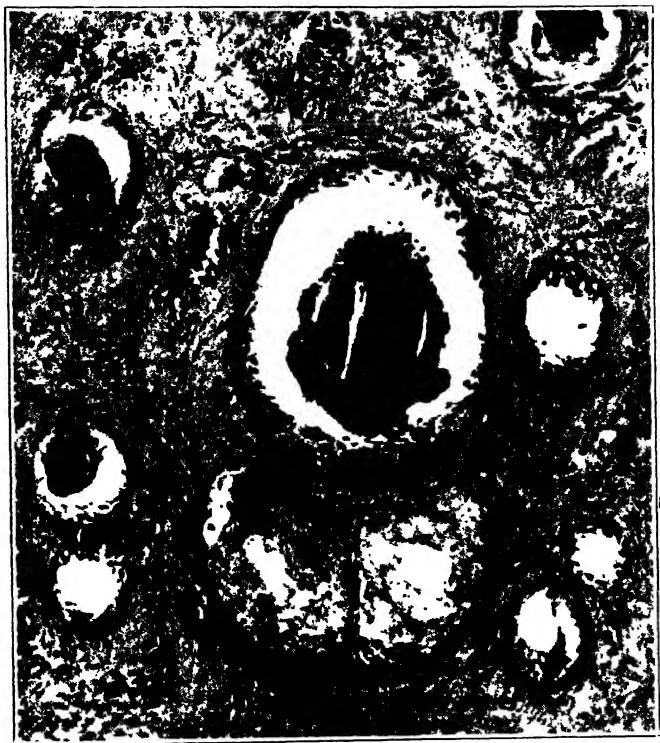


Plate 82.—Horizontal Section of Calf Skin through Sebaceous Glands.  
(After 5 weeks' contact with sterile lime-water)

Location: butt.

Thickness of section: 30  $\mu$

Stains: Van Heurck's logwood.

Daub's bismarck brown

Eye-piece: 5X

Objective: 8-mm.

Wratten filter: H-blue green

Magnification: 190 diameters.



**Plate 83.—Vertical Section of Thermostat Layer of Calf Skin.**

(After 1 day in 0.1-per cent pancreatin solution at 25° C.)

Location: butt.

Thickness of section: 30  $\mu$

Stains: Van Heurck's logwood,

Daub's bismarck brown.

Eyepiece: 5X.

Objective: 8-mm.

Wratten filter: H-blue green.

Magnification: 170 diameters.



**Plate 84.—Vertical Section of Thermostat Layer of Calf Skin.**

(After 1 day in 0.1-per cent pancreatin solution at 40° C.)

Location: butt.

Thickness of section: 30  $\mu$

Stains: Van Heurck's logwood,

Daub's bismarck brown.

Eye-piece: 5X

Objective: 8-mm.

Wratten filter: H-blue green.

Magnification: 170 diameters

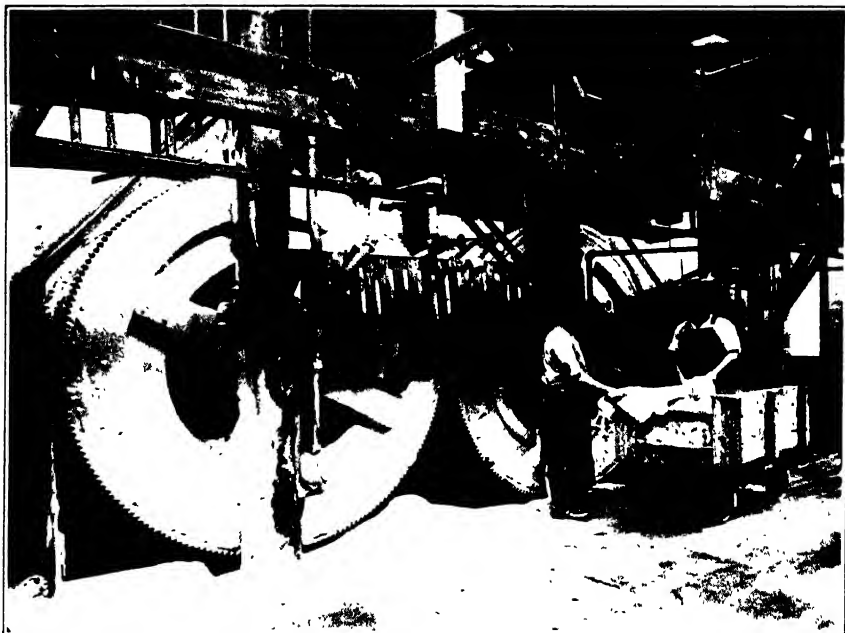


Plate 85.—Drums for Washing Limed Skins.

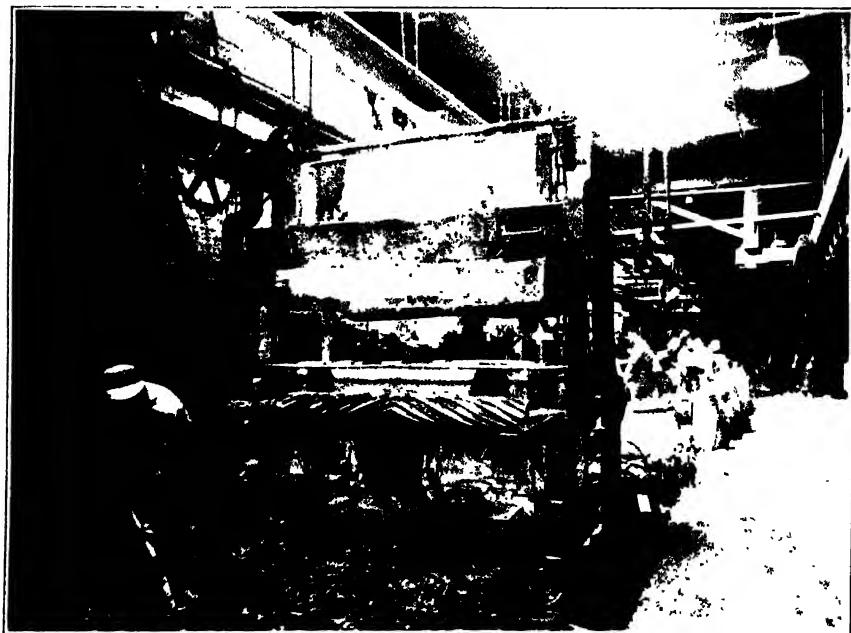


Plate 86.—Unhairing Machine.



it necessary to allow the liquor to act for a longer time in order to produce the desired ease of unhairing.

### Methylamine as an Unhairing Agent.

Among the protein decomposition products found in old lime liquors are methylamine,  $\text{CH}_3\text{NH}_2$ , and other amines, of higher molecular weight. Very recently McLaughlin, Highberger and Moore<sup>13</sup> discovered that primary alkyl amines, when added to a fresh lime liquor, greatly reduce the time required for satisfactory unhairing, while secondary or tertiary amines have little or no effect. Since amines are present in old, mellow lime liquors, they reasoned that the greater unhairing action of mellow lime liquors, as compared to fresh lime-water, is due to the primary amines they contain. The most promising amine experimented with is methylamine and it has proved to be an extremely powerful unhairing agent.

Merrill and Fleming, in the author's laboratories, checked the results of McLaughlin, Highberger, and Moore and found methylamine to be much more powerful in its action than ammonia. A pure 0.01-molar solution of methylamine at 27° C. was found to be capable of completely loosening the hair of calf skin in 18 hours, a 0.1-molar solution in only 1 hour, and a 0.5-molar solution in 30 minutes. To get the same effect in 18 hours with ammonia required a 0.15-molar solution, and in 1 hour a 5-molar solution. Methylamine thus appears to be from 15 to 50 times as powerful as ammonia. A 0.15-molar solution of ammonia has a pH value of 11.2; a 0.01-molar solution of methylamine a pH value of 10.6; a 0.1-molar solution a pH value of 11.6; and a 0.5-molar solution a pH value of 12.1. But the difference in action between methylamine and ammonia is probably not the effect of pH value. In saturated limewater a marked increase in unhairing action is noted upon the addition of only 0.001 mole per liter of methylamine, whereas a similar effect with ammonia requires the addition of about 5 moles.

If the price is ever reduced sufficiently, methylamine will probably enter the market as an unhairing agent. It appears to act more powerfully on the epidermis and less on the skin substance than sulfides. This is shown, to some extent, also in photomicrographs, published by McLaughlin and O'Flaherty,<sup>14</sup> illustrating the structural changes in skin taking place when unhairing with limewater alone and with limewater strengthened with sulfide or methylamine.

### Effect of Bacteria and Enzymes in Liming.

Where unsharpened lime liquors are used; that is, lime liquors containing no added sulfide or other accelerator of keratin hydrolysis, it is found that old liquors which have been used many times cause a more rapid loosening of the hair and less plumping of the skin than pure, saturated limewater. After each succeeding lot of skins has passed through the old lime liquor, it is prepared for the next lot merely by adding water to maintain the volume and enough lime to keep an appreciable excess over its solubility. These liquors become charged with decomposition products of the skin, including ammonia and amines, with lime salts, sulfides from the decomposition of hair, bacteria and enzymes and are usually called "mellow liquors."

Wood and Law<sup>38</sup> regarded the growth of bacteria in lime liquors as one of the principal factors in the production of mellowness. They examined an old lime liquor in which skins had been worked for 3 to 4 weeks and obtained a count of 50,000 bacteria per cubic centimeter of a type capable of developing in ordinary nutrient gelatin containing ammonia. They identified *Micrococcus flavus liquefaciens* and *B. prodigiosus*, both of which are known to produce proteolytic enzymes. The bacteria found on the roots of wool from the sweating process were found to be capable of growing in a liquid as alkaline as 0.05 normal. These appear to be similar to the bacteria commonly present in mellow lime liquors and Wood considers it highly probable that the unhairing action both in the sweat chamber and in mellow lime liquors is due to the same bacteria, not necessarily belonging to a single species.

Stiasny<sup>29</sup> also supposed that bacteria play an important rôle in old lime liquors. An untreated mellow lime liquor caused a loosening of the hair of calf skin in 24 hours and easy unhairing in 3 days, but in a test where chloroform was added to the same liquor to check bacterial action the liquor was not able to cause any loosening of the hair in 3 days and very little in 6 days. A portion of the untreated liquor was freed from ammonia by heating to 60° C. and passing carbon dioxide-free air through it for 4 hours. It then showed an unhairing power as great as before, but a lesser solvent action on the hide substance, from which Stiasny concluded that the unhairing action was due to bacterial action rather than to the ammonia ordinarily present in mellow liquors.

The view was formerly held that bacteria played a great part in unhairing and that bacteria and their enzymes were necessary to satisfactory unhairing in lime liquors. Schlichte<sup>28</sup> found that skin

previously sterilized by the Seymour-Jones process, with mercuric chloride and formic acid, could be unhaired easily after two weeks' contact with saturated limewater under sterile conditions. Wood and Law<sup>33</sup> raised the question as to the possibility of Schlichte's result having been influenced by the swelling action of the sterilizing solution, but the author has repeatedly demonstrated that fresh skin can be unhaired in pure limewater free from bacteria. An example of this is found in the work of Wilson and Daub,<sup>34</sup> described earlier in this chapter. They obtained easy unhairing of a fresh calf skin in saturated lime water at 20° C. after 5 days' contact and the lime liquor proved by actual test to be sterile.

Collett<sup>6</sup> made a series of studies of the bacterial counts of lime liquors, using media both at pH = 7.4 and at pH = 12.4, that of a lime liquor. At pH = 7.4, he obtained counts of 3000 per cc., or less, but with the media at pH = 12.4, no colonies developed, indicating that they could not grow at this pH value. The conclusion drawn was that some of the organisms can survive in a lime liquor, but cannot grow there. No increase in bacterial count was obtained when the lime liquor was used for longer periods. After a pack of skins was dumped into the liquor, the bacterial count might be initially as high as 2500 per cc. but it would gradually fall off to one-tenth of this before the pack was hauled out. The author compared the bacterial count of water used for soaking packs of skins with that of lime liquor used to unhaire the same skins. One pack of 350 domestic calf skins gave the first soak water a bacterial count of 243,000, the second soak water a count of 387,000, the third a count of 784,000, and the lime liquor following a count of only 7 per cc.

Collett<sup>7</sup> also studied the action of limewater on enzymes of many different types. The enzymes were added to saturated limewater, samples were removed at intervals, brought to pH values between 7 and 8 and the enzyme activity measured on appropriate substrates. In each case, the enzyme lost about 75 per cent of its activity after 15 minutes' contact with the limewater and all of it after one hour's contact.

Further proof that bacteria do not function in lime liquors under normal operating conditions has been furnished by McLaughlin, Rockwell and Blank.<sup>15</sup> They corroborated the finding of Wilson and Daub<sup>34</sup> that skins may be unhaired in fresh limewater without the aid of bacteria. They also showed that bacterial action on the skin before liming has a very great influence upon the liming process. A skin subjected to bacterial action before liming swells or plumps less

in the lime liquor and unhairs more readily than one limed in a perfectly fresh condition. This may be due partly to the bacterial action on the skin tissues rendering them more susceptible to alkaline hydrolysis and partly to the unhairing powers of the products of bacterial activity.

McLaughlin, Rockwell and Blank showed further that bacteria which do not form spores are readily killed by fresh limewater, but sporulating organisms are not killed, even in the presence of excess of undissolved lime, although their activity is inhibited. The antiseptic power of limewater is due, not only to its alkalinity, but also to its power to remove carbon dioxide which is necessary to the growth of the bacteria. The antiseptic action is appreciably influenced by temperature. Where bacterial activity in a skin has proceeded to an extreme degree before liming, bacteria may function to some extent during liming.

#### **Fresh vs. Mellow Lime Liquors.**

In a fresh lime liquor, the unhairing action is probably the direct action of alkali, or of sulfide followed by that of alkali, upon the epithelial cells of the Malpighian layer of the epidermis and upon the youngest cells lining the hair follicles, influenced but little, or not at all, by bacteria, enzymes, or ammonia.

In an old, mellow liquor, matters are complicated by a number of variable factors. The accumulation of calcium salts decreases the solubility of the lime. Both the increase in salt concentration and decrease in lime concentration result in considerably less swelling or plumping of the skin. The mellow liquor also contains considerable quantities of protein decomposition products having an influence on the unhairing action, such as ammonia and amines, whose actions are described above.

It has been thought that the sulfur accumulating in mellow lime liquors influences the unhairing action. McLaughlin, Highberger and Moore found that this sulfur had little influence upon the unhairing, but was actually a measure of the extent of the unhairing action. In the destruction of the keratinous matters of the epidermis by lime, sulfur is liberated. The amount of sulfur present, where only pure limewater is employed, is thus a measure of the amount of epidermal tissue hydrolyzed.

In extreme cases, where a mellow lime liquor has been allowed to become heavily charged with protein decomposition products and the pH value to fall considerably, due to lowered solubility of the lime

in the presence of accumulated calcium salts, it is possible that bacteria may become active and do considerable damage to the skins.

In the author's opinion, it is generally undesirable to use what are termed mellow lime liquors, whose compositions are very difficult to control. It is preferable to use fresh lime liquors with careful control of all concentrations, temperature, time, excess of undissolved lime, etc., rather than the old, mellow liquors with their variable contents of lime salts and protein decomposition products. The best values for the controlled variables in a fresh lime depend upon the kind of stock, its previous history and cure, the time and conditions of soaking, the nature of the processes to follow liming, and the specific values of certain properties desired in the finished leather. One tanner completes the liming of calf skins in one or two days, while another tanner takes a week; an investigation shows that the first tanner soaked the stock for four days and the second for only one. A tanner of heavy leather limes cow hides for two days in a single, fresh lime liquor, while another passes his stock through five liquors in succession over a period of a week; it is found that the first tanner maintains a temperature control of the liquor, while the second allows the temperature to vary with atmospheric changes. McLaughlin and Theis<sup>18</sup> have compiled some interesting data on the effect of varying the more important factors in liming, which should be consulted.

### Unhairing with Sulfides and Other Alkalies.

Pure solutions of sodium hydroxide and sodium sulfide quickly destroy the hair and epidermis when sufficiently concentrated. A 2-per cent solution of  $\text{Na}_2\text{S}$  at  $25^\circ\text{C}$ . will dissolve the hair and epidermis from the surface of a calf skin in about 2 hours, during which time only a comparatively small amount of collagen is destroyed. This treatment has been applied with considerable success to heavy hides, especially those which had previously been dried, and was a great help in speeding up the production of army leathers during the war. The hides were put into the sulfide solution, which was agitated by means of a paddle wheel. After several hours the hides were transferred to a solution of sodium bicarbonate or calcium chloride in order to stop the caustic action of the sodium sulfide. They were then washed and were ready for bating or tanning. The hair was completely dissolved from the surface of the hides in the sulfide liquor, but the action was so rapid that they had to be removed before the sulfide had diffused into them to the depth of the hair bulbs. As a result,

the hair bulbs were usually left in the hides intact, as could be shown by examining sections under the microscope, but this apparently did not lower the value of the leather in any way.

With this method of unhairing, it was found economical to use the same liquor for a number of consecutive lots of skins, adding just enough fresh sodium sulfide each time to maintain the necessary concentration. The liquors soon became heavily charged with protein decomposition products which are soluble in alkaline solution, but are precipitated by rendering the solution faintly acid. Kadish and Kadish<sup>10, 11</sup> made use of this fact in a scheme for recovering this nitrogenous matter as fertilizer. The waste liquors were run into a mixing chamber where they were reacted upon by sulfuric, sulfurous, or other acid. The precipitated nitrogenous matter was separated from the mother liquor and the hydrogen sulfide was recovered separately in such manner as to make the entire operation continuous. The fertilizer material obtained consists chiefly of keratose and its degradation products. This material will be discussed in detail in Chapter 10. Its isoelectric point is at  $\text{pH} = 4.1$ , so that controlling the pH value to this point will result in the greatest yield of keratose.

Barium hydroxide has been used for unhairing, but it is not only much more costly than lime, it is also more difficult to control, because of its greater solubility. With lime, it is safe to add to the liquor more than will dissolve, but with barium hydroxide the skins may be harmed by concentrations much greater than 0.02-molar, whereas solutions of about twice this strength result from using an excess.

Sodium hydroxide in concentrations from 1 to 4-molar has been used, but the skins seemed to suffer considerable damage. One method was to soak the skins in 4-molar NaOH for an hour or longer, which was sufficient to dissolve away all of the hair and epidermis. It was then transferred to a 10-per cent solution of sodium chloride containing enough sulfuric acid to neutralize all of the alkali carried over by the skin, which was then chrome tanned. The method is very rapid, but the leather was never of the same quality as that produced by unhairing with lime. At the same pH value, monacid bases seem to produce greater decomposition of protein matter than diacid bases, although this may be masked in some cases by specific ion effects. It was suggested by the author<sup>32</sup> that diacid bases should hydrolyze proteins to a lesser extent than monacid bases because of the higher valency of the cations. The swelling of protein jellies in alkaline solution is due to the pull of the cations of the protein salt upon the insoluble anions making up the jelly structure. The cations are in

true solution and tend to diffuse from the region of high concentration of the jelly to the region of lower concentration in the outer solution. But they cannot be separated very far from their insoluble anions upon which they exert a pull tending to drag them into solution. If this pull is sufficiently great, we might reasonably expect a breaking up of the units making up the jelly structure. A monovalent cation exerts its entire pull upon a single unit, whereas the pull of a divalent cation is divided between two units, making the tendency towards decomposing the protein only half as great.

Barium sulfide has found a use as a sharpening agent for lime liquors and also as an agent to dissolve hair when used in strong solutions. It has the advantage over sodium sulfide in that it produces less swelling or plumping of the skin. Mixtures of sodium sulfide and calcium chloride have been used rather widely to dissolve the hair from some kinds of stock. The low degree of solubility of the lime formed, especially in the presence of calcium salts, protects the skins against excessive swelling. Mixtures of calcium sulphhydrate and lime have also been found very satisfactory. The use of ammonium sulfide combines the specific effects of sulfide and ammonia, but is not suited for practical operations, at least in concentrated solutions, because of the objectionable odors.

It is not an uncommon practice in dewooling sheep skins to paint them on the flesh side with a paste made of a mixture of lime and sodium sulfide or of sodium sulfide and calcium chloride. The skins are then folded, wool side out, and left until the sulfide has diffused into the skins as far as the hair bulbs. When these are destroyed, the wool can be pulled or brushed out. As a rule, the skins are thrown over a beam and the wool is worked off by a beamster. The skins are then limed, washed, bated, and pickled, in which condition they may be kept until required for tanning. Sometimes the paste is made from lime and arsenic sulfide.

### Unhairing by Means of Acids.

In 1916, Mr. J. T. Wood sent the author a piece of calf skin which had been sterilized by the Seymour-Jones process. The formic acid had caused a loosening of the hair, which Mr. Wood says was marked in 8 days. Thuau<sup>31</sup> and Nihoul<sup>24</sup> had previously shown that sulfurous acid will cause a loosening of the hair of skins, if used in solutions that will prevent the swelling of the skin, as in the presence of salt.

Marriott<sup>17</sup> found that salted hide could be unhaired by immersion in 0.25-per cent acetic acid solution for 9 days.

In no case was the hair loosening by means of acid as satisfactory as can be obtained in alkaline solution. The acid seems to attack only the deepest layer of the epithelial cells of the Malpighian layer, leaving most of the epidermis intact, to be removed with the hair. It seems doubtful that acid will ever replace alkaline solutions for unhairing, especially since it has been demonstrated that acids attack collagen more readily than they do keratin.

### Unhairing with Stannous Salts.

The theory described above for the action of sulfides on hair led Merrill<sup>21</sup> to make the interesting prediction that reducing agents which are soluble in alkaline solution should be good unhairing agents. He chose stannous chloride for the test and found that it is, as was predicted, a remarkably good unhairing agent. His method was to place a sample of soaked calf skin, or of purified hair, in an alkaline solution of stannous chloride, to digest at a given temperature for a given time, and then to note the ease of unhairing or the extent of pulping of the hair.

Hair treated with a 1-per cent solution of stannous chloride for 48 hours appeared unattacked, but hair treated for 24 hours with the stannous chloride and then for 24 hours with saturated limewater was completely disintegrated. Hair treated with saturated limewater alone for 48 hours, of course, appeared unattacked. Hair treated with a 1-per cent solution of stannous chloride in saturated limewater for 48 hours was completely disintegrated. Stannic chloride solution, made by treating stannous chloride solution with chlorine until completely oxidized, had no action on hair.

The unhairing action of stannous chloride was tested by treating strips of soaked calf skin with solutions containing an excess of lime over its solubility and variable amounts of stannous chloride at 27.5° C. for 2 days. Some unhairing action was noticeable with as little as 0.05 gram  $\text{SnCl}_2$  per liter and the action was complete in 2 days where 0.5 gram per liter was used. With 5 grams per liter, the hair was completely disintegrated with the exception of the roots, which were somewhat protected by the skin. Per gram equivalent, stannous chloride was about as powerful an unhairing agent as sodium sulfide.

The effect of pH value was studied by adding varying amounts of sodium hydroxide to solutions containing 0.1 per cent of stannous



chloride and testing the unhairing action on calf skin. The pure solution of stannous chloride gave a strongly acid reaction and the skin was attacked. No unhairing action was observed until the pH value rose to 11.4. At pH = 11.8, complete unhairing was obtained in 2 days at 27.5° C. and the hair itself was attacked. The unhairing action of stannous chloride is manifest only in alkaline solution.

The experiments furnish evidence of the correctness of the theory of Atkin and Thompson,<sup>2</sup> described above. Since stannous chloride alone has no visible action on hair, but renders it more susceptible to attack by lime, support is given to Merrill's<sup>19</sup> theory of the action of sulfides that the reactions leading to unhairing take place in two stages. The use of stannous chloride as an unhairing agent is precluded by its high cost and the fact that it presents no working advantages over sodium sulfide or calcium sulphydrate, but the discovery of its action on hair is a valuable contribution to the theory of the mechanism of unhairing.

### Unhairing with Tryptic Enzymes.

In 1913, Röhm<sup>25</sup> described a process for unhairing and bating skins in one operation, involving the use of an alkaline solution of pancreatic enzymes. Since then, the tryptic enzymes of the pancreas have been listed as unhairing agents. As the action of enzymes in this method of unhairing becomes better understood, its use is increasing. In 1920, Hollander<sup>9</sup> described Röhm's process as having a number of advantages over the old system of liming, at least for certain classes of skins. According to his description, the skins are first soaked for 1 day in dilute sodium hydroxide solution and then transferred to a dilute solution of sodium bicarbonate to which the enzyme is added after the swelling due to the alkali has been counteracted. Twenty-four hours later the hair is completely loosened and can be rubbed off.

Wilson and Gallun<sup>35</sup> made a study of this method on calf skin. They found pancreatic enzymes to be good unhairing agents under certain conditions. At the concentrations they used, these enzymes acted under toluene at 40° C. At 25°, unhairing was obtained only in the absence of toluene, indicating that the unhairing action here was the result of bacterial activity. Later work in their laboratory, which will be described, showed that the enzyme will act at 25° under toluene, if present in sufficient quantity.

In view of the increasing commercial importance of this method and the scarcity of published data, it seems desirable to describe the

experiments of Wilson and Gallun in some detail. They made a preliminary examination by soaking pieces of thoroughly cleansed calf skin in 0.05-molar sodium hydroxide solution for 1 day, replacing the solution next day by 0.1-molar sodium bicarbonate solution, and 5 hours later transferring the pieces to a solution made by diluting 18 cubic centimeters of molar sodium hydroxide, 2.8 grams of monosodium phosphate, and 1 gram of U.S.P. pancreatin to 1 liter. The pH value of the solution was found to be 7.52 at 25° C., lying well within the range of optimum activity of this enzyme. Two experiments were run at a temperature of 25° C., but in one the solutions were left exposed to air, as would be the case in practice, while in the other they were covered with a layer of toluene to check bacterial action. After the pieces had been in the enzyme solutions for 24 hours, the hair of the pieces from the solutions exposed to air could be rubbed off with the greatest ease, leaving the grain surface clean and white, but that of the pieces from the solutions under toluene remained firmly fixed. This seemed to indicate that the unhairing action obtained at 25° was not due to the enzyme, but probably to proteolytic bacteria or their products.

Because of the doubt thus cast upon the rôle played by pancreatin in this method of unhairing, Wilson and Gallun carried the investigation further, paying particular attention to the action of pancreatin at 40° C., the temperature of its maximum activity. The studies were made upon pieces of fresh calf skin, about 5 x 3 inches, which had been thoroughly soaked and cleansed. Each experiment was carried out both at 25° and at 40° C. The action of the enzyme solution upon the skin in each test was compared with the action of a blank identical with the enzyme solution except for the fact that it contained no enzyme. This solution was prepared by diluting 18 cubic centimeters of molar sodium hydroxide solution and 2.8 grams of monosodium phosphate to 1 liter and all enzyme solutions were made by adding to it 1 gram of pancreatin per liter. The pH values did not vary more than 0.1 from the value 7.6 in any case. The enzyme solutions and blanks as well as solutions used for the pretreatment of the skin were all covered with a layer of toluene to check bacterial action. The results were checked on separate occasions with pieces of skin from different sources.

The effect of pancreatin upon skin not previously soaked in sodium hydroxide solution, or any other swelling agent, was studied first. After 24 hours of contact of skin and solution, little action was noticeable either at 25° or 40°, but after 48 hours the collagen fibers of

the skin in the enzyme solution at 40° began to dissolve very rapidly, the action proceeding from the flesh side, but there was no indication of the hair becoming loosened. On the other hand, the skin in the blank at 40° and those at 25° in both blank and enzyme solution still remained but little affected. It was evident that pancreatin has a more powerful solvent action upon the collagen fibers at 40° than upon the epidermis of a skin not previously swollen with acid or alkali. The time factor involved in the destruction of the collagen fibers is interesting. The action seemed to indicate that the fibers were coated with some material more resistant to tryptic digestion than the collagen beneath it. Possibly this supposed covering may be found to bear some relation to what Seymour-Jones<sup>27</sup> has called the fiber "sarcolemma."

In the next series of experiments, the pieces of skin were kept for 24 hours in 0.05 molar sodium hydroxide solution at 25° and 40° C., respectively. The solutions were then replaced by 0.1 molar sodium bicarbonate solutions of corresponding temperatures, and 5 hours later by the enzyme and blank solutions, in which the skins remained for 24 hours. The unhairing action in the enzyme solution at 40° was completely satisfactory, indicating that, at this temperature, pancreatin may be considered an unhairing agent for calf skin previously swollen in dilute sodium hydroxide solution. A very slight unhairing action was noticeable in the blank at 40°, evidently due to the previous treatment with alkali. No unhairing action could be detected in the blank or enzyme solution at 25°.

The preceding series of experiments was then repeated exactly, except that 0.05 molar hydrochloric acid solution was substituted for the alkali as the swelling agent. At 25° there was no visible unhairing action either in the blank or enzyme solution. In the hydrochloric acid solutions in the bath at 40°, the pieces of skin began to jelly; there was no further change in the piece transferred to the blank at 40°, but the piece put into the enzyme solution at 40° was quickly destroyed, the collagen passing into solution, leaving the epidermis and hair floating in the liquor. The opposite effects of acid and alkali upon the skin at 40° is interesting. 0.05 molar sodium hydroxide solution hydrolyzes the epidermis more rapidly than the collagen fibers, whereas 0.05 molar hydrochloric acid hydrolyzes collagen much more rapidly than it does the epidermis.

The experiment was repeated except for the fact that the pretreatment with hydrochloric acid was done at 25° and the digestion with pancreatin at 40°. After the skin had been in the pancreatin

solution for 24 hours, the hair was completely loosened, showing that the effectiveness of pancreatin as an unhairing agent depends upon the previous swelling of the skin, but regardless of whether the swelling is caused by acid or alkali. The fact that pretreatment with sodium hydroxide in the experiment with alkalies was done at 40° did not seriously influence the result for, when another piece of skin was soaked in 0.05 molar sodium hydroxide solution at 25° for a day and then in the pancreatin solution at 40°, the unhairing action was entirely satisfactory.

Experiments dealing with the action of pancreatin upon skins previously treated with ammonia were carried out exactly like those of the sodium hydroxide series, except for the replacement of the 0.05 molar sodium hydroxide solution by 0.50 molar ammonium hydroxide solution. The hair was loosened to some extent by the pretreatment with ammonia, more at 40° than at 25°. After the pieces had been in the blank and enzyme solutions for 24 hours, they all showed some unhairing action, but in no case was it entirely satisfactory. The degree of action might be given a very rough rating by calling that in the enzyme solution at 40° 75 per cent, that in the blank at 40° 50 per cent, and that in both blank and enzyme solutions at 25° 25 per cent. Evidently the pretreatment of skin with ammonia, which is itself an unhairing agent, does not assist the unhairing action of pancreatin nearly so much as pretreatment with materials whose action is primarily to swell the skin.

The question was raised that failure to get an appreciable unhairing action at 25° under toluene was due to an insufficient quantity of enzyme or of time of contact. Merrill and Fleming, working in the author's laboratory, tested this, using a more active enzyme preparation. The U.S.P. pancreatin used by Wilson and Gallun is sample no. 1 described in Table XXIX of the next chapter. Its activity on casein is 3 in Fuld-Gross units and its activity on keratose is 2.4. The sample used by Merrill and Fleming had an activity on casein of 17 Fuld-Gross units and a keratose value of 14.0, indicating that it was about 5.8 times as strong as the sample used by Wilson and Gallun.

Eight strips were cut from the neck of a calf skin after soaking and numbered 1 to 8. Strips numbered 5 to 8 were kept in water in the refrigerator at 7° C. until needed. Strips 1 to 4 were soaked for 24 hours in 0.05-normal NaOH solution and then for 5 hours in 0.1-normal NaHCO<sub>3</sub> solution. A phosphate buffer solution was made up consisting of 2.8 grams NaH<sub>2</sub>PO<sub>4</sub> per liter plus NaOH to make the pH

value equal 7.5. This was used to make up two series of four solutions each with concentrations of pancreatin of 0, 0.1, 1.0, and 10.0 grams per liter, respectively. One strip of skin was put into each solution in a bottle under toluene and the bottles were kept in a constant temperature bath at 25° C. Each strip was examined daily and the time required for satisfactory unhairing noted. The results are as follows: With pretreatment with NaOH and NaHCO<sub>3</sub>, unhairing was complete in 1 day with 10 grams of enzyme per liter, 3 days with 1 gram, 6 days with 0.1 gram, and not in 12 days with no enzyme. Without pretreatment, unhairing was complete in 5 days with 10 grams of enzyme per liter, while it was not complete in the other tests in 12 days.

These tests show that pancreatic enzymes are unhairing agents, if used in sufficiently high concentration, even under toluene and without special pretreatment, at 25° C. Pretreatment with NaOH and NaHCO<sub>3</sub> lessens the time and concentration of enzyme required. The action of the enzyme is assisted by bacterial activity where the bacteria are allowed to thrive.

### Combined Unhairing and Elastin Removal by Enzymes.

Wilson and Gallun<sup>35</sup> extended their investigations to an examination of the effect of the enzymes upon the elastin fibers of the skin. Pieces of skin were taken from the various experiments after the enzymes had acted upon them. These were imbedded, sectioned, stained, and mounted for examination, as described in Volume II.

In the experiments at 25° C., it was concluded that the unhairing action was the result of bacterial activity and not of pancreatin, since it was checked by the addition of toluene. Plate 83 shows a section of the skin which unhaird easily at 25°. The epidermis is disintegrated and the hair loosened, but the elastin fibers remain undissolved and show in the upper half of the picture as darkly stained threads, running nearly horizontally. Plate 84 shows a section of the skin unhaird by the enzymes under toluene at 40°. Not only is the epidermis destroyed and the hair loosened, but the elastin fibers have been completely dissolved. It seems that where the active agent in unhairing is the enzyme, the elastin fibers are removed at the same time, but where the unhairing is brought about chiefly by bacterial activity, the elastin fibers are not removed. This work was not repeated at the higher concentrations or activities of enzyme.

In the unhairing experiments where the skin from the enzyme

solutions at 40° C. had not previously been swollen with acid or alkali, microscopic examination showed that all of the elastin had been dissolved away from the flesh side of the skin in 24 hours, but none from the region just under the epidermis. The hard corneous layer of the epidermis had apparently acted as a membrane impermeable to the enzyme. In the ordinary methods of unhairing, such as liming, the unhairing agent acts upon the cells of the Malpighian layer, which lie between the corneous layer and the derma. The impermeability of the corneous layer to the enzyme explains why the pancreatin did not attack the Malpighian layer and loosen the hair. In acid or alkaline solutions, the corneous layer swells considerably and is thereby rendered more permeable. It is also attacked by the enzyme, when in the swollen condition, as shown by the fact that no corneous layer could be found in the sections examined.

Skins prepared for unhairing and scudding by means of pancreatin solutions are unhaired on a machine, scudded on the beam, and then washed, after which they are ready for tanning without further treatment. Skins from lime liquors are unhaired, scudded, washed and then either bated, delimed, drenched, or pickled before tanning. Some tanners put the skins directly into old vegetable tan liquors without giving them one of these treatments, but the tan liquor then becomes a deliming agent and has little value other than that of removing lime.

Apparently anything that will hydrolyze the newly formed cells of the epidermis without injuring the rest of the skin is a satisfactory unhairing agent. Lime owes its popularity to the safety attending its use. Its limited solubility makes it possible to maintain a constant hydroxide-ion concentration at about 0.022 mole per liter simply by using an excess. This concentration is high enough to retard putrefaction considerably and yet not great enough to injure the skin itself, since the solute is a diacid base. It is entirely possible, however, that the popularity of lime will wane when some of the newer methods of unhairing reach a higher stage of development.

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## Chapter 10

### Bating

Perhaps the most curious of all the processes involved in making leather is that of bating. For centuries this was one of the mysterious processes of the tannery and, until comparatively recently, it was easily the most disgusting. It has been so completely enshrouded in secrecy, jealously guarded, that it has not been possible to trace its origin. However, the process as handed down by the generations past is still an unpleasant memory to many tanners living today. It consisted in digesting the limed and unhaired skins in a warm infusion of the dung of dogs or fowls until all plumpness had disappeared and the skins had become so soft as to retain the impression of thumb and finger when pinched and sufficiently porous to permit the passage of air under pressure. When hen or pigeon manure was used, the process was called bating, and when dog dung was used, it was called puering, but the term bating is now applied to the process generally, regardless of the materials used. The difference in terminology naturally disappeared with the advent of artificial bating materials.

A common method for treating light skins was to put them into a vat filled with a liquor containing about 100 grams of dog dung per liter, kept at a temperature of 40° C. by means of steam. A paddle wheel kept the liquor and skins in motion. During the action, the skins gradually lost the plumpness acquired in the lime liquors and became soft and raggy. The completion of the process was determined by the attainment of a certain degree of flaccidity, which the workmen could judge only after long experience. Hen or pigeon manure was sometimes used for light skins, but was more commonly applied to heavy hides because it penetrates more rapidly than dog dung, due apparently to the fact that it contains also the urinary products, especially urea.

It is possible to omit the bating process by treating the limed and unhaired skins with acid solutions to delime them and then to tan them, but leather so made does not have the fine appearance of bated stock. The effect of bating upon the finished leather is to



give a smoother and cleaner grain surface, which has so great an effect upon the selling value of the leather as to make bating a practical necessity for the finer light leathers.

Many attempts have been made to ascertain the mechanism of bating whereby the skin is improved in quality. The greatest pioneer work in this field was initiated by J. T. Wood, whose investigations, coupled with practical developments by O. Röhm and others, have led to the almost complete replacement of the obnoxious dungs by pancreatic enzymes. In his book, Wood <sup>24</sup> says: "When learning the trade as an apprentice every fault in the leather was attributed to this part of the work, and the troubles and miseries of the 'puer shop' first caused me to take up the study of puering. I was determined to know the causes underlying the process. Puering is not only a filthy and disgusting operation, but is prejudicial to health, and in the nature of it is attended by more worry and trouble than all the rest of the processes in leather making put together."

Wood found the mineral matter of dungs to consist chiefly of the sulfates, chlorides, carbonates, and phosphates of sodium, potassium, ammonium, and calcium, and some silica. The most important organic constituents seemed to be the bacteria, enzymes, cellulose materials, and fats. He found both peptic and tryptic enzymes, a rennin, an amylolytic enzyme, and a lipase. Since the bate liquor is usually faintly alkaline, it seemed likely that trypsin was active in the process and it was later shown that this enzyme does produce some of the effects of dung upon the skin. Wood also isolated from dog dung a species of *B. coli* which was found to yield an enzyme capable of acting upon the skin like trypsin.

The modern bating material now almost universally used consists of a mixture of pancreatic enzymes and ammonium chloride or other buffer salt designed to maintain the pH value of the bate liquor between the values 7.5 and 8.5. A common procedure for calf skins is to take them after scudding, wash them for an hour with running water in an open drum like that pictured in Plate 85, and then to put them into a paddle vat containing the bate liquor. The concentrations of enzyme and of buffer salts, the temperature of the liquor, and the time of digestion required are interrelated variables which depend also upon the conditions employed in soaking and liming, as well as upon the kind of skin. In general, less enzyme is required the higher the temperature, up to 40° C., and the longer the time of digestion. Or, for a fixed concentration of enzyme, the higher the temperature, the less the time required. The relation of these variables

will be discussed in detail later. When the skins have been paddled about in the liquor for the required time, they are hauled out and sent to be tanned.

In former times, the endpoint of the bating process was determined by feeling the skins. Plate 87 shows a bate master examining the skins to determine whether or not the digestion has gone far enough, basing his decision upon whether or not the skins have become sufficiently flaccid. The author has found that flaccidity is not a reliable guide as to the endpoint in bating, although it does serve to safeguard the skins against bacterial damage, which does not become appreciable until after the pH value has dropped to the range where the skin becomes flaccid. The best guide as to the optimum conditions for bating is the appearance of the skin and leather, although it has the disadvantage of operating only after the bating process is over. With a tannery in continuous operation and all liquors chemically controlled, it is not difficult to determine the conditions of bating required to give the finest appearance to the grain surface and then to adhere rigidly to those conditions.

In some light leather tanneries, it is customary to sort the skins after bating according to suitability for one kind of tannage or another. Plate 88 is a typical view of this operation.

### Mechanism of Bating.

Many investigations have been carried out to determine the nature of the reactions occurring during the bating of skins, which are responsible for their later behavior and appearance. In the author's laboratories studies of the bating of calf skins have been in progress for many years, the results of which are summarized in this chapter. They will be discussed under seven headings indicative of the nature of the important reactions involved in bating: (1) falling; (2) regulation of pH value; (3) deliming; (4) bacterial action; (5) removal of elastin fibers; (6) digestion of keratose; and (7) hydrolysis of collagen.

### Falling.

The one property which all of the various types of bating materials have in common is that of reducing the degree of swelling of the protein constituents of the limed skin, which action is known to the trade as *falling*. Indeed it would have been practically impossible for any artificial preparation to pass as a bate that did not have this

property, because the degree of flaccidity of the skin was the accepted measure of the nearness to completion of the bating process.

It will be apparent from the discussion of the swelling of protein jellies given in Chapter 5 that the degree of falling of a skin must be a function of hydrogen-ion concentration and also of the concentration of neutral salts.

Wilson and Gallun<sup>29</sup> measured the degree of plumping of calf skin as a function of pH value by means of their method, which is described in Chapter 9. Pieces of unhaired skin, each about 2 centimeters square, were cut from the butt of a calf skin so as to insure the greatest degree of uniformity of structure. These were freed from lime by washing in a 12-per cent solution of sodium chloride containing a small amount of hydrochloric acid, and then neutralized in cold, saturated sodium bicarbonate solution. They were then washed and bated by keeping at 40° C. for 24 hours in a solution containing 0.1 gram of U.S.P. pancreatin, 2.8 grams of monosodium phosphate, and enough sodium hydroxide per liter to give a pH value of 7.7. Microscopic examination showed that this procedure removed all of the elastin fibers. The pieces were then washed in cold, running tap water, having a pH value of 8, for 24 hours. They were then kept in distilled water in the refrigerator at 7° C. until used for the tests. The condition in which the skin existed in this state was taken as a standard, as it was found to be easily reproducible.

A series of 24 large reservoirs of test solutions was prepared, each having a final concentration of tenth-molar phosphoric acid plus the amount of sodium hydroxide required to give the desired pH value as determined by the hydrogen electrode. A range of pH values from 4 to 11 was covered.

In each test a piece of skin in standard condition was placed in the Randall and Stickney thickness gauge described in Chapter 9. The gauge reading in every case was taken exactly five minutes after dropping the plunger onto the piece of skin. This was called the initial gauge reading. The skin was then shaken with water to bring it back to its natural shape and then put into 200 cubic centimeters of standard buffer solution of the desired pH value and kept in a thermostat refrigerator at 7° C. so as to reduce to a minimum any tendency towards putrefaction. After 24 hours, each solution was replaced by fresh buffer solution. After 4 days more, there being practically no change taking place in the pH values of the solutions, it was assumed that equilibrium was established and the pieces were removed and their thicknesses measured again. The results are given

in Table XXII. The ratio of the final to the initial gauge reading is a measure of the degree of plumping of the skin and this is plotted as a function of the pH value in Fig. 61.

TABLE XXII

UNHAIRC'D CALF SKIN IN CONTACT WITH BUFFER SOLUTIONS OF DIFFERENT pH VALUES.

Gauge Readings in mm. (Average of Duplicates)			pH Value of Solution at 20° C.	
Initial	Final	Ratio *	Initial	Final
1.421	2.729	1.92	3.96	3.97
1.205	1.885	1.56	4.14	4.17
1.269	1.431	1.13	4.47	4.49
1.439	1.296	0.90	4.78	4.79
1.489	1.305	0.88	5.08	5.07
1.299	1.161	0.89	5.29	5.27
1.347	1.239	0.92	5.57	5.57
1.388	1.306	0.94	5.78	5.72
1.212	1.263	1.04	6.04	6.08
1.225	1.270	1.04	6.29	6.29
1.391	1.478	1.06	6.48	6.42
1.248	1.343	1.08	6.69	6.68
1.435	1.514	1.06	6.96	6.88
1.292	1.362	1.05	7.08	7.00
1.379	1.415	1.03	7.41	7.41
1.413	1.385	0.98	7.68	7.62
1.393	1.407	1.01	7.97	7.89
1.515	1.520	1.00	8.42	8.44
1.428	1.427	1.00	8.56	8.50
1.253	1.343	1.07	9.03	9.13
1.258	1.377	1.09	9.59	9.64
1.219	1.388	1.14	10.00	9.98
1.240	1.621	1.31	10.47	10.51
1.289	2.206	1.71	11.06	11.08

\* This ratio is a measure of the degree of plumping of the skin.

The significance of these two points of minimum plumping has been discussed in Chapter 5. By comparing Fig. 61 with Fig. 12, it will be seen that the plumping of calf skin varies in much the same way as the swelling of gelatin with change of pH value. Apparently collagen undergoes a change of form, possibly an internal rearrangement, in passing from an acid to an alkaline solution and the two points of minimum represent the isoelectric points of the two forms.

The degree of plumping at any point between 4.5 and 9.0 is relatively so small that the skin would pass as completely bated, if judged solely by its *fallen* condition. Wood, who was probably the first to apply the hydrogen electrode to tannery liquors, observed that the pH value of fresh dung bate liquors varied from about 4.7 to 5.4, whereas the bating of a pack of skins raised it to points lying between 6.4 and 8.4. In a lime liquor, which has a pH value of about 12.5,

the skin is very plump and rubbery. But when it is brought into equilibrium with a liquor having a pH value lying between 4.5 and 9.0, it becomes fallen and flaccid.

Merrill<sup>8</sup> studied the effect of time, temperature, and concentrations of enzyme and of ammonium chloride upon the rate of falling of calf

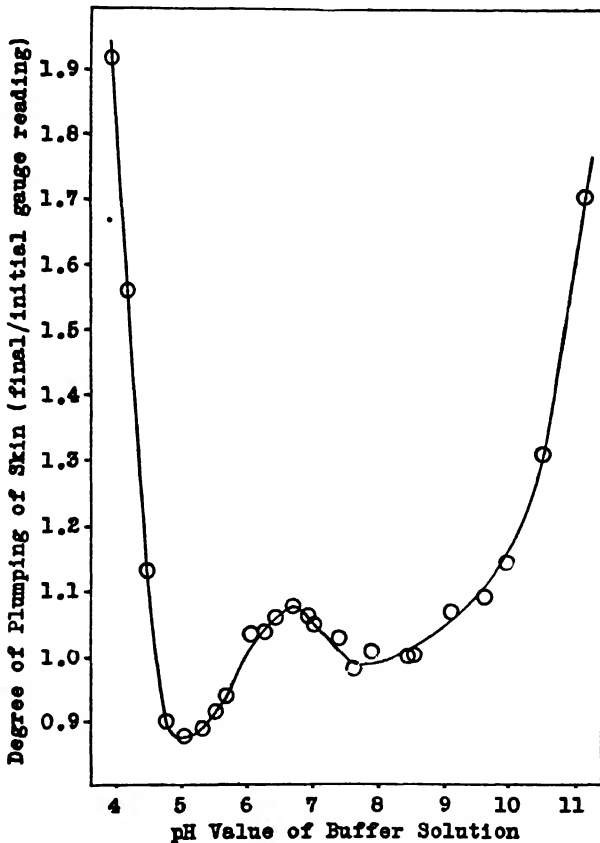


FIG. 61.—Showing the two points of minimum plumping of calf skin.

skin in bating. He used the Wilson-Gallup method, described above, but recorded falling in terms of the percentage decrease in initial thickness of the skin, as the bating progressed.

Before studying the temperature effect, it was necessary to determine the effect of ammonium chloride concentration, the effect of enzymes, and the time required to attain equilibrium at some one temperature, in order to fix experimental conditions. It was found

that, as the concentration of ammonium chloride was increased, the degree of falling also increased until enough ammonium chloride was present to react with all the lime. Further additions of ammonium chloride had very little effect upon pH value, and consequently none on the extent of falling. The presence of enzymes increases the degree of falling, but only when they are present in quantities much greater than are ever used in bating, the effect being due, seemingly, to the actual destruction of a considerable portion of the skin.

The effect of temperature on the rate of falling was studied by running series of experiments at different temperatures, making the time of digestion the independent variable in each series. The results of these tests are given in Table XXIII. The concentration of ammonium chloride used lies in the range where further increases of concentration do not much affect the extent of falling. No enzyme was added in these experiments.

TABLE XXIII  
INFLUENCE OF TEMPERATURE ON RATE OF FALLING  
(0.864 gram ammonium chloride per liter)

Time Digested Hours	7° C.	Decrease in Initial Thickness at			35° C.
	Percent	25° C. Percent	30° C. Percent	Percent	
0.5	9.9	22.7	23.0	25.1	
1.0	15.8	22.0	29.7	26.7	
2.0	15.5	27.2	28.2	28.7	
4.0	20.3	...	...	28.6	
17.0	22.2	29.6	29.4	28.7	

The speed with which complete falling is attained increases with the temperature. Thus, at 30° and 35° C. virtually complete falling was reached in from 1 to 2 hours; at 25° C. a somewhat longer time was required; and at 7° falling was not complete in 17 hours. The difference in rate is not very marked, however, except in passing from moderate to low temperatures.

To determine the effect of temperature on the extent of falling at equilibrium, a series of experiments was carried out at temperatures increasing from 10° C. by increments of 5° C., the time of digestion being 18 hours in all cases. The results of these tests (Table XXIV) show that the extent of falling increases somewhat with increasing temperature. Here again the effect is not appreciable except when rather wide variations in temperature are in question.

In general, it may be concluded that temperature has little effect on falling, when changes of a few degrees are involved. If, however,

TABLE XXIV

INFLUENCE OF TEMPERATURE ON FALLING (18 HOURS)

	(0.864 gram ammonium chloride per liter)						
Temperature, ° C. ....	10	15	20	25	30	35	40
Decrease in thickness, per cent	22.9	21.8	23.8	24.9	22.6	25.4	24.4

it were desired to change from a bate at 30° C. to one 20 degrees lower, a somewhat longer time of bating would be required to effect complete falling, aside from other considerations.

A number of materials not containing any enzyme have appeared on the market under the name of bating materials which serve merely to reduce the pH value of the limed skins to the region of minimum plumping. The value of the fallen condition is readily apparent for skins which are to be tanned in vegetable tan liquors. Tannins diffuse only very slowly through swollen skin, but when the skin is in a fallen condition, the tannins are enabled to diffuse more rapidly into the spaces between the fibers, greatly hastening complete penetration of the skin. The preparation of materials of this kind is a very simple matter. It is only necessary to incorporate a buffer material with one which will tend to lower the pH value of the limed skin to a final value of about 8. Among the materials used for this purpose are boric acid, ammonium chloride, weak organic acids and materials yielding acids by fermentation, and acid sodium phosphate. The author observed five successive lots of skins pass through an artificial bate liquor containing sodium phosphate, which was entirely uncontrolled, and 0.5 was the greatest deviation in pH value from the normal value of 8.0 during the entire period of operation. Where it is desired only to bring the skins into a fallen condition, the process can be carried out very effectively using only sodium phosphate and the occasional addition of hydrochloric acid to maintain a pH value of about 8. For heavy leathers, or leathers for special purposes, this may be sufficient, but for light leathers a much finer appearance of the grain surface is obtained by the use of pancreatic enzymes.

### Regulation of pH Value.

Although the degree of plumping of a skin is a function of the hydrogen-ion concentration, the action of a bate liquor in lowering the pH value of limed skin has an importance independent of the question of plumping. Nearly 80 per cent of the bated weight of a skin is due to water, or rather bate liquor. Even though the skin may be washed, the water will assume a pH value depending upon

the substances held in combination with the skin. This adhering solution will therefore have an effect upon the tan liquor into which the skins are put. If the pH value of this adhering solution is very variable, difficulty will be experienced in vegetable tanning because the rate of tanning, the rate of diffusion of the tan liquor into the skin, the color value of the tan liquor, and its tendency to oxidize are all functions of the pH value. Keeping constant the pH value of the solution adhering to the skins entering the tan liquors is a factor of great importance and one which made the old dung bates almost a necessity to the tanner who had no other way of controlling the pH value. The actual pH value, within limits, was probably of less importance than keeping it constant at some arbitrary value, which could be met by establishing conditions in the tan yard to correspond.

### **Deliming.**

There are many statements in the literature that would indicate that the chief function of bating is to free the skins from lime. In fact, lactic acid has been sold as a bating material, with this object in view. In using a dung bate, Wood found from 3 to 6 per cent of lime, calculated as calcium oxide on the dry skin, before bating and only from 0.5 to 0.9 per cent after bating and all of this appeared to be present as neutral salt.

However, the author has repeatedly observed the changes in lime content of skins bated with trypsin and ammonium chloride. On the dry basis and calculating as  $\text{CaO}$ , the percentage of lime averaged about 3 per cent after scudding, about 2 per cent after washing, before bating, and about 2 per cent after bating. During the bating, the lime present as hydroxide or in combination with protein simply became carbonated and remained in the skin as calcium carbonate. After a pickling operation, which followed, the lime content had dropped to 0.6 per cent, present chiefly as calcium sulfate. Where the bate liquor merely carbonates the lime, without removing it, it would be incorrect to refer to the bating process as a means of deliming. Where the lime is not removed in bating, it is usually removed by the acids in pickling, drenching, or the first tan liquor into which the skins are put.

### **Bacterial Action.**

In dung bates and in trypsin bates where the same liquor is used to bate a number of successive lots of skins, it is probable that the result is influenced by bacterial action. Plate 95, from a paper by



Wood,<sup>35</sup> shows a typical plate culture on gelatin of a dung bate liquor in actual use. A great many bacterial counts of bate liquors have been made in the author's laboratories. One interesting series is from a liquor used to bate 8 successive lots of calf skins. The counts after using, each time, in millions per cubic centimeter were 93, 370, 590, 920, 1330, 1110, 770, and 630. After reaching a maximum of 1,330,000,000 per cc., the count began to fall off. Apparently the bacteria were being killed off by their own excrement.

Becker<sup>1</sup> isolated 54 varieties of bacteria from dog dung and studied the actions of many of them upon skin. He found one, which he called *B. erodiens*, capable of producing a falling action of limed skin similar to that of the dung bate itself. An artificial bacterial bate was developed independently by Wood in England and by G. Popp and H. Becker in Germany, but they later joined forces and perfected the artificial bate known as erodin, which consists of a nutrient material to which a pure culture of *B. erodiens* is added before using. This material has been used on a commercial scale and found to be a satisfactory substitute for dung for some kinds of leather.

Since *B. erodiens* does not secrete tryptic enzymes, Wood has suggested adding to it bacteria obtained from the roots of wool in the sweating process which secrete a mild form of proteolytic ferment. The susceptibility of erodin liquors to become contaminated by foreign bacteria presents an obstacle to any very widespread increase in their use. In using erodin, Wood has observed that the fresh liquor usually has a pH value of about 6.6 and this increases to about 7.3 during the bating operation.

Cruess and Wilson<sup>2</sup> isolated 10 varieties of bacteria from pigeon dung and found that the falling of limed skins could be brought about by pure cultures in dilute skim milk. If the bating operation were unduly prolonged, the skin proteins became hydrolyzed, but they found that danger from this source could be minimized by using a liquor containing 0.5 per cent of glucose. They pointed out that the glucose was decomposed into acids which checked bacterial action and assisted in the removal of lime from the skin.

### Removal of Elastin Fibers.

Wood<sup>36</sup> separated the enzymes from dog dung by precipitation from solution with alcohol and showed that the enzymes, in conjunction with ammonium compounds, were capable of bating skins. In view of the fact that the bate liquor was alkaline, it seemed pretty

certain that trypsin must be the principal enzyme acting. Wood and Law<sup>37</sup> later showed that there were at least five different enzymes present in dog dung, as follows:

1. A peptic enzyme resembling stomach pepsin.
2. A tryptic enzyme resembling pancreatic trypsin.
3. A rennin (coagulating enzyme).
4. An amylolytic enzyme.
5. A lipase.

Where a skin contains an abundance of fat cells, the lipase probably exerts an important function in hydrolyzing and emulsifying the fats.

In 1908 Röhm<sup>14</sup> patented the use of the enzymes of the pancreatic juice and ammonium salts as a bating material. This mixture now known as oropon has come into wide use and has largely supplanted the dung bates formerly used.

Recently there has been a concerted effort to determine just what part is played by pancreatin in the bating process. Attention was first centered upon the action of trypsin upon the elastin fibers. As a measure of the elastin content of skin, Rosenthal<sup>18</sup> used the per cent of nitrogenous matter that could be rendered soluble by tryptic digestion. By this method he found that bating with oropon reduced the elastin content of calf skin from 10.36 to 0.31 per cent, calculated on the dry basis. The author's later investigations of the bating process by means of the microscope, however, indicate that Rosenthal's method of determining the elastin content of skin gives false results. Apparently a large portion of the matter included as elastin was derived from the other protein constituents of the skin or their hydrolytic products.

Upon examining a dung bate liquor used to bate sheep grains, Wood found that nitrogenous matter had been dissolved equivalent to only one per cent of the total protein matter of the skins. As nearly as can be judged from microscopic observations, this represents approximately the percentage of elastin present in the skin.

Seymour-Jones<sup>19</sup> also suggested that the function of bating is the removal of the elastin fibers of the skin. In collaboration with J. T. Wood, Seymour-Jones carried out an interesting experiment on the bating of sheep skin. The "flywing" grain of a sheep skin was split from the main body of the skin, called simply flesh for convenience, and both grain and flesh were cut into halves along the backbone. One grain and one flesh were bated with pancreol, a pancreatin preparation similar to oropon, while the other halves were delimed with acetic acid,

but not bated. All four pieces were then tanned with sumac. There was comparatively little difference between the bated and unbated flesh halves, but the grain samples were very different from each other. The bated grain was soft and even, with the hair-holes clean and clear, but in the unbated grain the hair-holes appeared to be glued up and the surface had a rough, contracted appearance. He concluded that elastin present in the region of the grain membrane must be digested before tanning in order to produce a satisfactory grain surface, but that the bating of the skin under the grain is not only unnecessary, but often undesirable.

Wilson and Daub<sup>27, 28</sup> studied the effect of trypsin upon the elastin fibers of calf skin under the microscope. The enzyme they used was purchased under the name U.S.P. pancreatin and is listed in Table XXIX as sample no. 1. A specimen for examination was cut from a calf skin after liming, unhairing, scudding and washing, but before bating. The skin was then bated for 24 hours at 40° C. in a solution containing 0.1 gram per liter of this enzyme, 0.02 mole of phosphoric acid and enough sodium hydroxide to bring the pH value to 7.5. Plate 89 shows a section of the skin before bating and Plate 90 a section after bating. The elastin fibers stain more deeply than the collagen fibers and so appear in Plate 89 as very dark masses of threads forming a thick band just under the grain surface. The magnification here is not sufficiently great to show each individual elastin fiber. Another layer of these fibers appears near the flesh boundary of the skin. The main body of the skin contains no elastin fibers excepting those surrounding blood vessels, nerves, and muscles. Plate 90 shows that bating has completely removed the elastin fibers.

Krall<sup>9</sup> claimed priority in discovering, by means of the microscope, that elastin is removed in bating. His experiments, performed at the University of Geneva from 1914 to 1916, proved that the elastin fibers of skin can be entirely removed by digestion in an infusion of dog dung at 40° C. His photomicrographs show that the action of dog dung on the elastin fibers is exactly the same as the action of pancreatin observed by Wilson and Daub. Krall's important paper was unfortunately buried in a private bulletin.

By examining skins under the microscope, Wilson and Daub could detect no other removal than that of elastin and, naturally, concluded that the chief action of the enzyme in bating was upon the elastin fibers. These fibers, being present primarily in the thermostat layer, would be expected to influence the appearance of the grain surface of the leather, and they probably do. But it was shown later by

Wilson and Merrill<sup>30</sup> that the enzyme acts also and more energetically upon an important constituent of limed skin that is not visible under the microscope in the sections examined by Wilson and Daub because it is soluble. This material, called keratose, is a degradation product of keratin and its removal from certain kinds of skin in bating is very much more important than the removal of elastin. However, the removal of the elastin fibers results in a softness and extensibility of the grain that may be desirable for some kinds of leather. The keratose is so much more readily hydrolyzed by trypsin than elastin that it is possible to bate away practically all of the keratose before the elastin has been measurably affected.

In studying the digestion of elastin by trypsin, Wilson and Daub prepared sections of calf skin, before and after bating with trypsin, and stained them as described in Volume II. By comparing the two sections in each case under the microscope, a rough estimate was made of the per cent of the elastin removed by the treatment.

For each series of experiments, Wilson and Daub cut a piece of limed and un haired calf skin into strips about 2 x 0.5 inches. There is a small, but appreciable, difference in time required for complete removal of elastin from skins of different thickness and for this reason care was exercised in selecting all strips for any one series from the same part of the same skin, so as to have them all as nearly identical as possible. Each strip was put into 500 cubic centimeters of liquor, a volume large enough to prevent the skin from seriously altering the concentration of the liquor. The liquors were all put into dark brown bottles to shield them from the light and were kept in a large Freas thermostat for the stated lengths of time at  $40^{\circ} \pm 0.01^{\circ}$  C., the optimum temperature for most enzyme actions.<sup>8</sup>

Every liquor contained 0.02 mole per liter of added phosphoric acid to act as a buffer, in addition to the enzyme, and the potassium hydroxide required to give the desired hydrogen-ion concentration. The pH value of each liquor was determined both before and after the digestion period by means of Hildebrand electrodes and a Leeds and Northrup potentiometer, excepting where it was proved by previous test that the results obtained by the Clark and Lubs series of indicators were sufficiently accurate. Except for the more strongly acid and alkaline solutions, the change in pH value during digestion was practically negligible. Estimates of the per cent of elastin removed were made on the basis of removal from the grain layer only. In some cases all of the elastin was removed from the grain layer before half of it was removed from the flesh layer. Since the shaving operation

removes practically all of the flesh elastin, its removal in bating is of little importance.

As a rule, a preliminary series covering a very wide range was run, followed by a second series covering only the active range of the enzyme. A third series was usually run as a check.

*Effect of pH Value.* It is well known that pH value is an important factor in determining the rate of digestion by enzymes. Using 0.1 gram of pancreatin per liter and digesting for 24 hours, complete

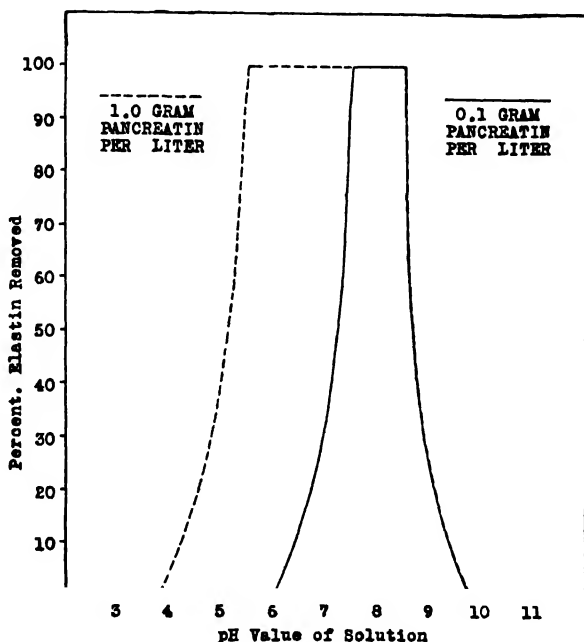


FIG. 62.—Removal of elastin fibers from limed calf skin as a function of hydrogen-ion concentration. Time of digestion, 24 hours; Temperature, 40° C.

removal of elastin from the skin was obtained only between the pH values 7.5 and 8.5. A portion of the pancreatin was put into a collodion sac and dialyzed against running tap water in a dark room for 16 hours and used in a duplicate series in such quantity as to represent 0.1 gram per liter of the original pancreatin. The results were identical with those obtained with the undialyzed enzyme. A series was then run in which the concentration of pancreatin was increased to 1.0 gram per liter. Complete removal of elastin was obtained between the pH values 5.5 and 8.5. The results of the two series, which are shown in Fig. 62, were carefully checked to insure their accuracy. The

per cent of the total elastin which was removed is plotted against the pH value of the solution taken after digestion and cooling to 25° C.

The peculiar relation of the curves to each other is significant. They nearly coincide at all pH values above 7.5, but at 6.0 the stronger solution is still capable of digesting all of the elastin in 24 hours, while the weaker one has apparently entirely lost its elastin-digesting power. When an enzyme has been found to have different optimum pH values with different substrates, it has been supposed that the effect of the hydrogen-ion concentration upon the substrate has been the determining factor. But here we have the same enzyme and the same substrate, with a change in the optimum range due merely to a change in concentration of the enzyme.

An explanation is suggested by the work of Northrop,<sup>11, 12</sup> who has shown that the activity of an enzyme solution is not necessarily a function of the apparent total enzyme concentration, but that a portion of the enzyme may be inactivated by combining with peptone or other foreign matter. He has pointed out further that the extent of the formation of addition compounds between protein and enzyme depends upon the concentration of protein ion, which in turn is a function of the hydrogen-ion concentration. If some protein other than elastin is responsible for the inactivation of a portion of the enzyme, we should expect such action to be a minimum at the isoelectric point of this protein.

After bating, the strips of calf skin were all carefully examined for the "bated feel," which apparently bears no relation to elastin removal, but corresponds to a condition of minimum swelling of the skin proteins. The only strips passing this test were those from liquors having pH values between 6.1 and 9.8, with the point of greatest flaccidity at about 8. This value evidently corresponds to the second point of minimum plumping of calf skin found by Wilson and Gallun and shown in Fig. 61. It is worthy of note that at 40° C. Wilson and Daub found no indication of a point of minimum except at 8. On the basis of the theory of the existence of two forms of collagen, discussed in Chapter 5, it would appear that at 40° Wilson and Daub were dealing only with the form stable at higher temperatures and pH values and whose isoelectric point appears to be at 7.7.

The following explanation is therefore suggested tentatively. At a pH value of 7.7, practically all of the enzyme is left free to attack the elastin, but as the pH value is decreased and the concentration of collagen cation correspondingly increased, more and more enzyme is removed from the field of action by combining with it. In the

weaker enzyme solution at pH = 6 practically all of the enzyme is in combination with collagen, whereas in the stronger solution the excess of enzyme is still sufficient to digest elastin. The reason for coincidence of the curves on the alkaline side is probably that 0.1 gram of enzyme was more than sufficient to saturate the surface of the elastin fibers. Any amount in excess of this would be without effect, but would act as a reservoir to be drawn upon when some of the enzyme was withdrawn from the field of action.

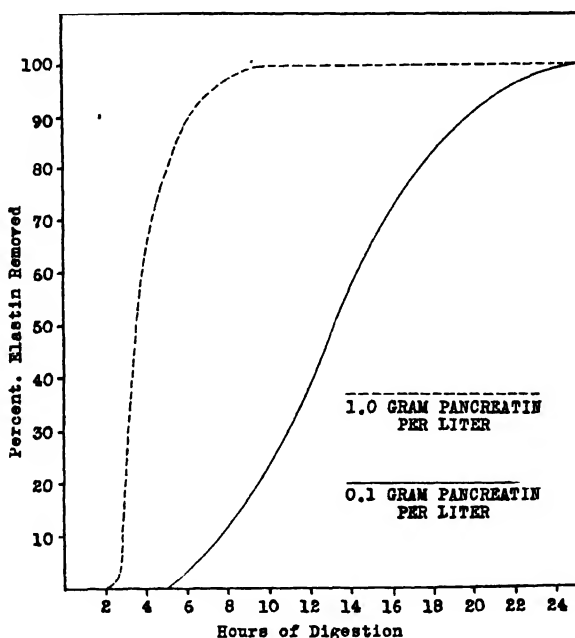


FIG. 63.—Removal of elastin fibers from limed calf skin as a function of time of digestion. Temperature, 40° C.; pH value, 7.6.

It is interesting to compare the optimum pH values for tryptic digestion found by other investigators: <sup>3</sup> for albumose Michaelis and Davidsohn <sup>10</sup> found 7.7; for casein Sherman and Neun <sup>20, 21</sup> found 8.3, while Long and Hull <sup>6</sup> found 5.5 to 6.3; and for fibrin Long and Hull <sup>6</sup> found 7.5 to 8.3. The total range of 5.5 to 8.3 corresponds closely to the range found by Wilson and Daub, 5.5 to 8.5, for complete removal of elastin by the more concentrated enzyme solution.

At pH values less than 3.0 there was a marked destruction of the collagen fibers, evidently due to acid hydrolysis, and the strips

were much swollen and rubbery, but no removal of elastin could be detected.

*Effect of Time of Digestion.* Two series were prepared, one in which the concentration of enzyme was 0.1 gram per liter and the other in which it was 1.0. All members of both series were otherwise identical. The pH value of each liquor was brought to 7.6 and this did not change during digestion. Each strip of calf skin was kept in a separate bottle. The bottles were removed from the thermostat at fixed intervals during 24 hours.

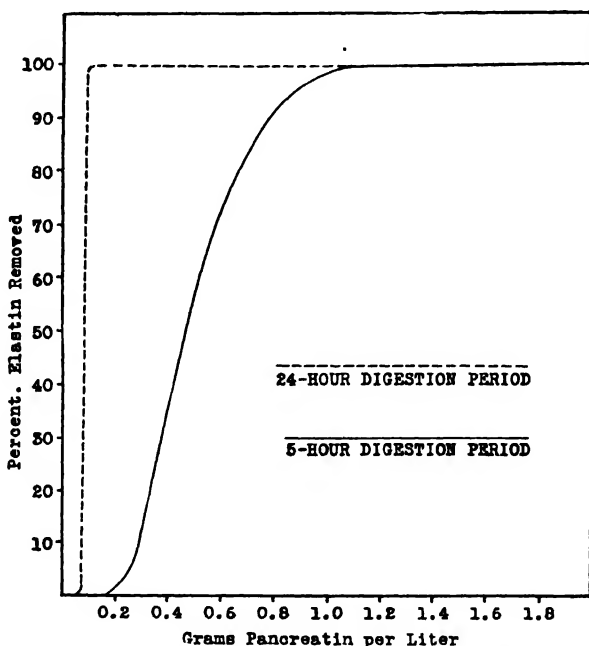


FIG. 64.—Removal of elastin fibers from limed calf skin as a function of concentration of enzyme. Temperature, 40° C.; pH value, 7.6.

Complete removal of the elastin was effected by the stronger enzyme solution in 6 to 8 hours, but in the weaker solution 24 hours were required. The progress of the digestion is shown in Fig. 63. The time required to start the digestion, 2 hours for the stronger and 5 hours for the weaker solution, was apparently the time required for the enzyme to diffuse into the region of the skin containing the elastin fibers. As will be seen from Plate 90, these begin about 0.1 millimeter below the grain surface.

*Effect of Concentration of Enzyme.* Two identical series of solu-



tions were prepared in which the individual members differed only in concentration of pancreatin. One series was kept in the thermostat for 5 hours and the other for 24 hours. The results are shown in Fig. 64 and furnish a study in economy. Complete removal of elastin is effected by 0.1 gram of pancreatin in 24 hours or by 1.1 grams in 5 hours.

*Effect of Concentration of Ammonium Chloride.* A study of bating would not be complete if it did not include the effect of am-

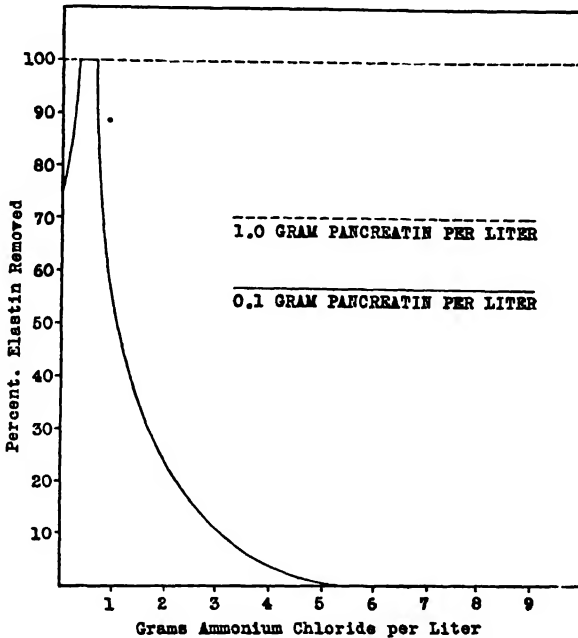


FIG. 65.—Removal of elastin fibers from limed calf skin as a function of the concentration of ammonium chloride. Time of digestion, 24 hours; Temperature, 40° C.; pH value, 7.6.

monium chloride, one of the most abundant constituents of commercial bating materials. Aside from its use as a filler, it has been assumed to be beneficial in removing lime from the skins and tending to maintain a slight alkalinity favorable to tryptic digestion. Two series of solutions were prepared in which the concentration of enzyme was 0.1 and 1.0 gram per liter, respectively. To each successive member of each series increasing amounts of ammonium chloride were added and the pH values of all members were brought to 7.6. The time of digestion was 24 hours. The results are shown in Fig. 65.

In working with very dilute enzyme solutions, a distinct activating effect was noted upon the addition of 0.5 gram per liter of ammonium chloride, while larger amounts showed an inhibitory effect. With thin calf skin the activating effect was not detectable with the solution containing 0.1 gram per liter of enzyme after a 24-hour digestion period, because all of the elastin was removed without adding any ammonium chloride. In order to show the activating effect in these experiments, strips from heavier skins were used, which require a somewhat longer time for complete removal of elastin under fixed conditions. The activating effect of 0.5 gram of ammonium chloride per liter and the inhibitory effect of greater concentrations are very marked. It is also important to note that the effect of ammonium chloride can be entirely overcome by a sufficient excess of enzyme.

This behavior of ammonium chloride is interesting in view of the finding of Thomas<sup>24</sup> that potassium bromide in concentrations of 0.0 to 0.1 mole per liter has an inhibitory effect upon the action of malt amylase, but in greater concentration has an activating effect.

At concentrations greater than 50 grams per liter the ammonium chloride exerted a destructive action upon the collagen fibers.

### **Distribution of Elastin Fibers in the Skins of Different Animals.**

It is well appreciated by tanners that skins of different animals and of animals of different ages must be treated differently in bating, as well as in other processes. It has been noted, for example, that the bating of a cow hide is less effective than the bating of a calf skin under the same conditions. The reason for this will be made apparent by comparing Plates 91 and 92, both of which were photographed at exactly the same magnification. They represent the upper portions of the skins taken after liming, unhairing, scudding, and washing, but before bating. Plate 91 is from a full grown cow hide, while Plate 92 is from a young heifer calf skin. It will be noted that the older skin has relatively fewer elastin fibers, although they extend into the skin to a greater absolute depth. This greater depth necessitates leaving the skin in the bate liquor for a longer time to permit the enzyme to reach the most deeply seated fibers, but, on the other hand, there is less reason for removing elastin fibers from the heavier skin, because they are relatively fewer.

Plate 93 shows the elastin fibers of a sheep skin before bating and Plate 94 those of a hog skin. The elastin fibers of the hog skin are very sparsely scattered; the heavy band of elastin fibers passing

obliquely upward to the right, across the center, is apparently associated in some way with the erector pili muscle, which it surrounds.

Plates 91, 92, 93, and 94 should be compared with Plates 12, 28, 30, and 33, respectively, which show sections taken from the same skins when fresh.

### Effect of Elastin Removal.

It is extremely difficult to measure quantitatively the effect of enzymes in bating upon the properties of the finished leather because there is a bigger difference in properties of individual skins treated in an identical manner than the effect upon the properties attributable to the enzymes. This is a most important fact in this study. It was responsible for a number of misconceptions prevalent when investigations in this field were first begun. Certain tests on a few calf skins showed no practical differences whether an enzyme was used in bating or not. It was only when the differences between individual skins were eliminated, by using hundreds of skins for each test, that the real effect of enzyme action became apparent. In all of the later experiments in the author's laboratories, 700 skins were used for each test, 350 being subjected to a standard procedure and 350 to the procedure being studied. In a given test, it might be found that 300 skins of the standard pack were of high grade, so far as appearance of the grain was concerned, and 50 of lower grade; in the test pack 200 skins might be of high and 150 of lower grade. This sort of finding has been common in the bating experiments and any number of repetitions of the test bear out the original finding.

It is easy to see why experiments on a smaller scale might be misleading. In the test used as an example, if one skin had been picked for the test, by the law of chance it would have been one which turned out well with either bating procedure. Had it been split down the backbone into sides and one side bated each way, it would have indicated that the procedures gave identical results. Or a poor skin might have been put through the better procedure and a good skin through the poorer one and the findings would have been the reverse of the truth. Only when one procedure is decidedly damaging to a skin can a reliable test be made with a single skin. In our experiments on bating, many tens of thousands of calf skins have been used.

When a comparison is made between skins which have been bated with enzyme and skins treated with the same liquors, but without enzyme, there is always a decided difference in appearance of the grain surface in favor of the enzyme bated skins. But it has been

found possible to bate skins with so little enzyme that the elastin fibers are not visibly attacked and yet get the fine appearance of the grain characteristic of bated skins. During the early stages of tanning, the effect of elastin removal is pronounced. The surface layers of the skin tan more rapidly than the fibers in the interior and there is a tendency for the grain surface to expand temporarily to a greater extent than the rest of the skin. Where the elastin fibers are present, they tend to prevent this expansion and the result of the tension produced is a slightly harsh feel, although the grain appears tight and smooth to the eye. Where the elastin fibers have been completely removed, the grain expands noticeably in the tan liquors, giving the skin temporarily a wrinkled appearance, although the grain feels very soft and silky, in contrast to the skin containing elastin fibers. By the time the skins are fully tanned, this difference has practically disappeared. In the finished leathers, the appearance of the grain is practically the same whether the elastin fibers are removed or not.

The result is that we have found the removal of elastin unnecessary for calf skin, although it is necessary to bate with enzyme. Wood<sup>35</sup> states that it is not necessary, nor even desirable, to remove all of the elastin in bating, but that it is sufficient for the elastin fibers to be broken up or weakened, in order that the desired suppleness may be obtained. Röhm and Haas<sup>16</sup> have suggested that a more important function of bating consists in dissolving out remains of unorganized material constituting the scud, the insoluble material derived from the epithelial tissues. Marriott<sup>7</sup> also believes that it is important to remove materials other than elastin in bating. For leathers in which an extremely soft and extensible grain is desired, bating to remove all of the elastin fibers is undoubtedly beneficial, but for most calf leathers, at least, the really important function of the enzyme in bating seems to be the digestion of keratose.

### Digestion of Keratose.

From a study of the bating of calf skins on a very extensive scale, Wilson and Merrill<sup>30</sup> were led to suspect that the most valuable work done by the enzyme in bating is the hydrolysis of keratose. In some cases the rough and dirty appearance of the grain surface of skins which had not been bated with enzyme appeared to result from the precipitation of nitrogenous matter in the thermostat layer of the skin. It was, of course, well known that a heavy precipitate of degradation products of hair and other keratinous matter is formed

when old lime liquors are titrated with acid. Because of the abundance of epithelial tissue in the thermostat layer, it is evident that there must be a considerable quantity of degraded keratin in this layer after liming. If it is not removed, it will be precipitated by the tan liquors in the thermostat layer, with consequent damage to the appearance of the grain surface. As will be shown later, it has now been pretty well established that the primary purpose of the enzyme in bating is to break down this degraded keratin, or keratose, into products more easily washed out of skin and which do not precipitate upon addition of acid.

*Preparation of Standard Keratose.* The important material to be studied consists of those decomposition products of keratin which are soluble in neutral or alkaline solution, but precipitated upon acidification. The term "keratose" has been limited to this material in consequence of which the keratose content of the prepared solutions may be defined as the weight of material precipitated by the addition of acid to the point of maximum precipitation.

The keratose used in these experiments was prepared by digesting thoroughly cleansed calf hair with ten times its weight of 2 *N* sodium hydroxide for 18 hours at 25° C. The resulting solution was treated with hydrochloric acid to reduce the pH value to 8, at which the keratose is still soluble. The solution was filtered and then acidified to pH = 4, at which point the keratose was precipitated as a voluminous, white mass, such as is seen when titrating a used lime liquor with acid. The precipitate was allowed to settle in tall cylinders and washed repeatedly by decantation. Finally, it was redissolved by adding sodium hydroxide to make the pH value of the solution equal 8. This solution was preserved with a small amount of thymol and kept in the refrigerator until used.

*Isoelectric Point of Keratose.* The isoelectric point of keratose was measured by the pH value causing maximum precipitation. Keratose solution, containing approximately 2 per cent by weight of keratose, was mixed with an equal volume of the powerful citrate-phosphate-borate buffer solution described by Northrop<sup>13</sup> and 8 volumes of water. One hundred cubic centimeter aliquots were pipetted into 50-cc. portions of acetic acid solutions of different concentrations to give pH values covering the range of maximum precipitation. The precipitate formed in each case was filtered off, dried, and weighed. The maximum amount precipitated in any test was taken as the total keratose content and the percentage precipitation in the other tests was calculated from this. Two entirely independent series of tests were run

and both are plotted in Fig. 66. The point of maximum precipitation is fairly sharply defined at  $\text{pH} = 4.1$ .

An attempt was made to cover a greater range than that shown in Fig. 66, but at  $\text{pH}$  values just outside this range the precipitate was so slimy as to be practically unfilterable. At  $\text{pH}$  values still further removed from the range no precipitate was formed at all. It is rather important to note that the isoelectric point of keratose corresponds closely to the  $\text{pH}$  value of many tanning solutions.

*Effect of pH Value.* In each test the keratose and enzyme solutions were mixed with the citrate-phosphate-borate buffer solution,

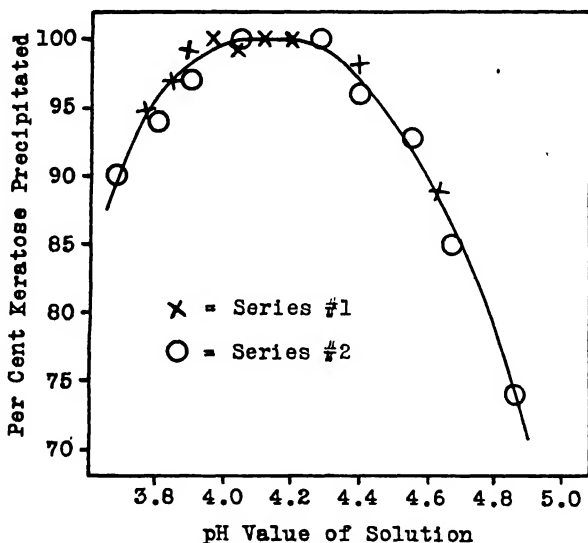


Fig. 66.—Isoelectric point of keratose indicated by point of maximum precipitation

described by Northrop,<sup>13</sup> to which hydrochloric acid or sodium hydroxide had been added to produce the desired  $\text{pH}$  value in the digestion mixture. A stock buffer solution was made with 30.85 grams of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 30.35 grams  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 5.5\text{H}_2\text{O}$ , and 10.3 grams  $\text{H}_3\text{BO}_3$  per liter. A liter of digestion mixture contained 100 cc. of the stock buffer solution and enough acid or alkali to give the desired  $\text{pH}$  value. The object of the buffer solution was to maintain constancy of  $\text{pH}$  value during the digestion, and electrometric measurements made before and after each digestion showed that this was accomplished. It was also demonstrated that the presence of the buffer material did not introduce any complications, since series run

without added buffer gave results more erratic, but otherwise similar in value to those obtained with buffer material. The digestions were made at 40° C. The pancreatin used is listed as sample no. 1 in Table XXIX.

At the end of the digestion period, the undigested keratose was precipitated by the addition of enough acetic acid-sodium acetate mixture to bring the pH value to 4.1, filtered off, dried, and weighed on a tared filter paper.

Fig. 67 shows how the hydrolysis of keratose varies with pH value in the presence and absence of pancreatin. Subtracting the values in

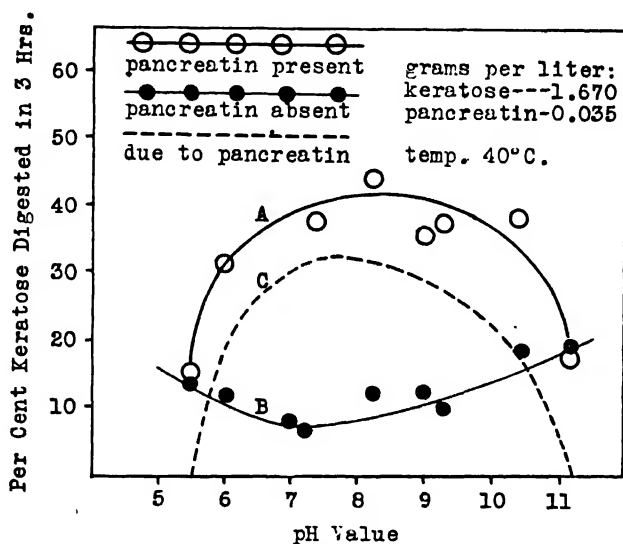


Fig. 67.—Effect of pH value on digestion of keratose by pancreatin.

curve B from those in curve A, we get curve C, which represents the extra hydrolysis due to the added enzyme. At pH values below 5.5 and above 11.2, the enzyme is without effect, while it shows its optimum action at pH = 7.9. The range of comparatively great activity extends from about 7.5 to 8.3, which is the range ordinarily found in bate liquors.

*Effect of Time of Digestion.* The progress of the digestion of keratose by pancreatin with time is illustrated in Fig. 68. For comparison, a blank and a solution of a typical commercial bate are included. The curves are typical of protein hydrolysis by enzyme and the curve for the blank shows that during the first 24 hours practically all the digestion is due to the enzyme.

*Effect of Concentration of Enzyme.* With enzyme concentration the only variable, it was found that the time required to hydrolyze a given fraction of the keratose is inversely proportional to the concentration of enzyme. This is shown strikingly by the straight lines in Fig. 69, where the reciprocal of the number of hours required to digest 20, 30, and 40 per cent of the keratose, respectively, is plotted against the initial concentration of the enzyme.

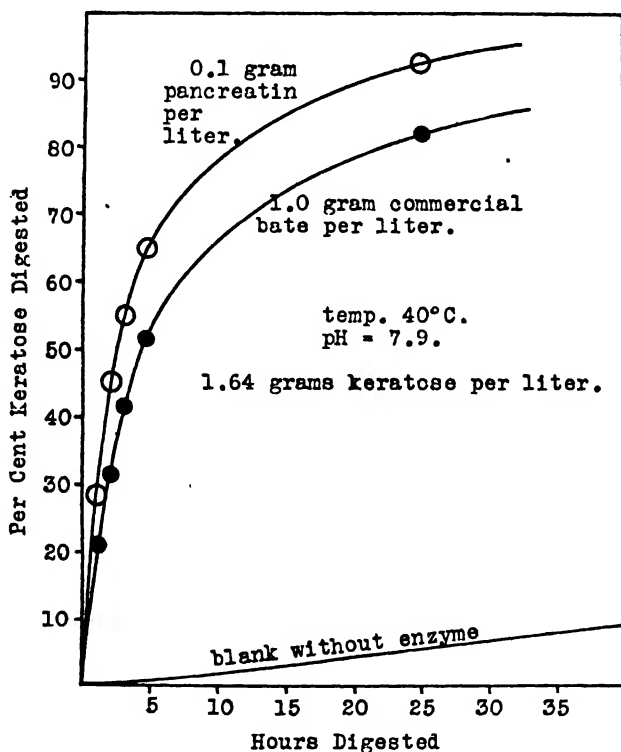


FIG. 68.—Digestion of keratose by enzymes as a function of time.

*Effect of Concentration of Keratose.* For a given initial concentration of enzyme, the rate of digestion increases with the value for the initial concentration of keratose. This is indicated in Fig. 70, where the time required to hydrolyze a given fraction of the keratose is plotted against the initial concentration of keratose. Where 2 grams per liter of keratose are present, 0.8 gram is hydrolyzed in 135 minutes, but where 4 grams per liter are present, 0.8 gram is hydrolyzed in only 93 minutes.



*Effect of Temperature.* Merrill<sup>8</sup> has made a number of important measurements and observations of the effect of temperature on bating. The effect of temperature on the rate of a chemical reaction is usually measured by determining the velocity constant of the reaction at different temperatures. The quotient of the reaction velocity constants at temperatures 10 degrees apart is commonly taken as the temperature coefficient of the reaction for the particular interval in question. For

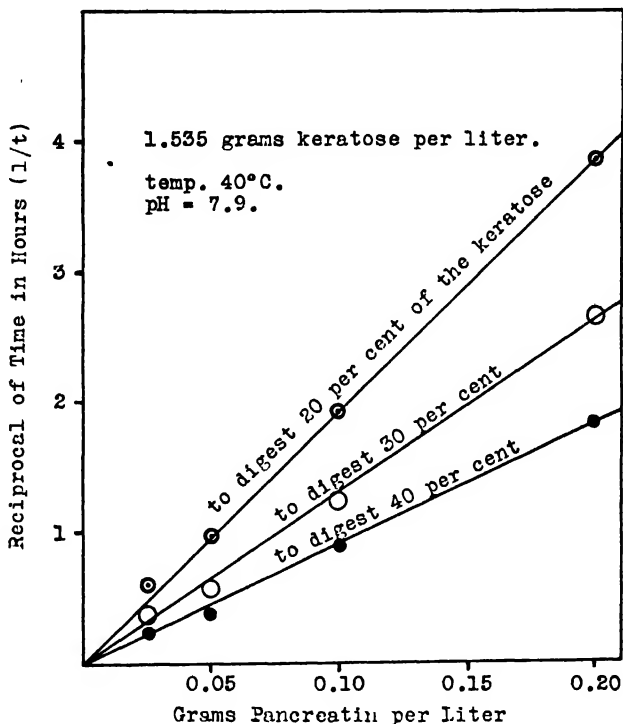


Fig. 69.—Rate of digestion of keratose as a function of concentration of pancreatin.

most reactions the temperature coefficients, measured in the neighborhood of room temperatures, are between 2 and 3.

It is not possible to obtain satisfactory values for the velocity constant of the hydrolysis of keratose in the presence of trypsin, because, generally speaking, the course of the reaction cannot be expressed in terms of any of the familiar equations of chemical kinetics. The reason for this probably is that the enzyme itself is destroyed during the progress of the reaction, so that the rate of decomposition

of the substrate falls off more rapidly than would be predicted from the mass law. To measure the rate of hydrolysis of keratose it is, therefore, necessary to measure the time required for the digestion of some specified fraction of the total keratose present, under definite conditions of concentration of substrate and enzyme.

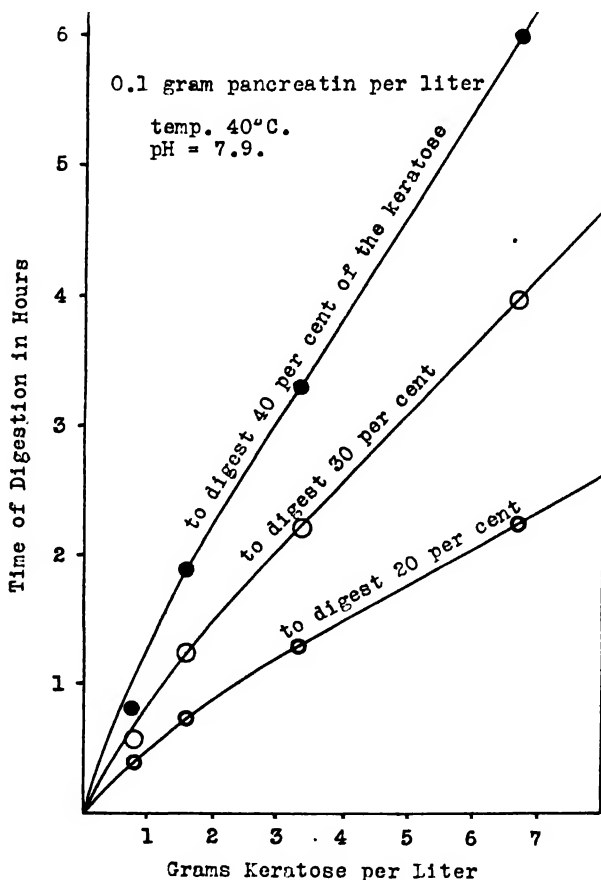


FIG. 70.—Time required to digest a given fraction of keratose as a function of initial concentration of keratose.

In the Wilson-Merrill method<sup>30</sup> for measuring the activity of an enzyme upon keratose, the results are expressed in terms of  $1/hg$ , where  $h$  is the number of hours required for the digestion of 40 per cent of 2.0 grams of keratose, contained in 1 liter of solution of pH 8.0, at 40° C., and  $g$  is the concentration of enzyme in grams per liter. In making the determination,  $g$  is fixed arbitrarily and  $h$  is measured by

determining the fraction of the total keratose digested at different time intervals, plotting the percentage digested against time and determining the time required for 40 per cent digestion by interpolation. The percentage of keratose digested is determined by precipitating undecomposed keratose at its isoelectric point, filtering and weighing, and subtracting the weight found from that taken initially. For further details of the method, the papers of Wilson and Merrill already referred to may be consulted.

To determine the effect of temperature on the rate of digestion of keratose, the obvious procedure is to measure  $1/hg$  at several different temperatures, employing the same enzyme. This course, however, involves certain difficulties, as the following considerations will show.

The net effect of temperature on any enzyme reaction is the resultant of two independent and distinct effects. Like most reactions, the digestion of a protein by an enzyme increases in velocity with rising temperature. On the other hand, the enzyme undergoes a spontaneous inactivation, which is more rapid the higher the temperature. Whether an increase in temperature will accelerate or retard an enzyme action depends upon which of these two processes is most affected. At lower temperatures the change in the rate of spontaneous inactivation with temperature is relatively small. At higher temperatures it is relatively large. The result is that the velocity of most enzyme reactions passes through a maximum at about 40° C.

The inactivation of an enzyme naturally becomes more apparent the longer the period of digestion, from which it follows that the relative rate of digestion at two different temperatures will be determined in magnitude, and even in sign, by the fraction of substrate the digestion of which is taken as the end point of the reaction. At 50° C., for example, the hydrolysis may start much more rapidly than at 40°, and the time required for 20 per cent digestion may be much shorter at the higher temperature. After a short time, however, the enzyme may have lost so much of its activity at 50° C. that the reaction practically stops, so that a very much longer time is required to digest 50 per cent of the substrate at 50° C. than at 40° C.

Different enzyme preparations undergo inactivation at very different rates, the less powerful preparations being, in general, more resistant to temperature inactivation than more highly purified materials. From this it follows that temperature coefficients found for one enzyme specimen cannot safely be used for others without further tests.

These conclusions are fully supported by the following experimental results:

The rate of digestion of keratose was determined at 5-degree in-

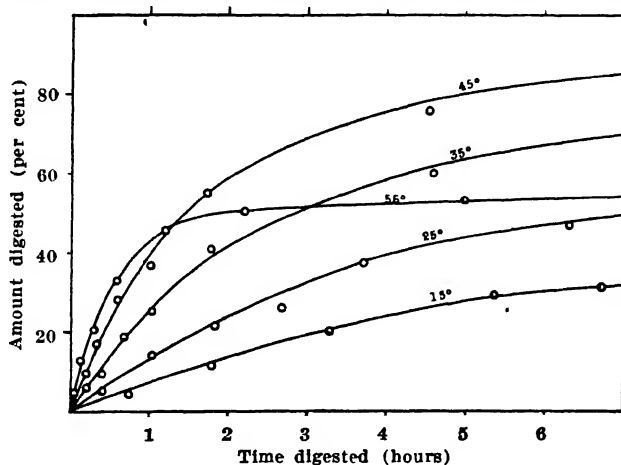


FIG. 71.—Rate of solution of keratose (keratose, 2 grams per liter; trypsin No. 6, 0.02 gram per liter) at different temperatures.

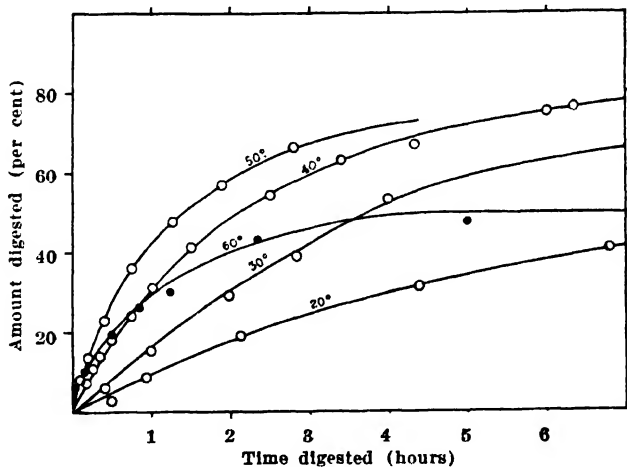


FIG. 72.—Rate of digestion of keratose (keratose, 2 grams per liter; trypsin No. 6, 0.02 gram per liter) at different temperatures.

tervals from 15° to 60° C. The enzyme used was sample no. 6 listed in Table XXIX. The per cent digested was plotted as a function of time in Figs. 71 and 72 and the time required for the digestion of 20, 30, 40, and 50 per cent of the total substrate was read off from the curves for each temperature.  $1/hq$  was calculated for the different

fractions of total digestion for each temperature. The values so obtained are collected in Table XXV (columns 2 to 5). Temperature coefficients were calculated by dividing  $1/hg$  for each temperature into  $1/hg$  for the temperature 10 degrees higher. These coefficients are collected in Table XXV (columns 6 to 9).

TABLE XXV

EFFECT OF STAGE OF REACTION ON APPARENT TEMPERATURE COEFFICIENT OF DIGESTION OF KERATOSE BY TRYPSIN <sup>a</sup>

Temperature ° C.	$1/hg$ Digestion				$(1/hg)_T + 10 / (1/hg)_T$			
	20 Percent	30 Percent	40 Percent	50 Percent	20 Percent	30 Percent	40 Percent	50 Percent
15	14.9	8.3	5.1	3.2	2.1	2.1	2.4	2.6
20	21.3	11.9	7.6	5.8	1.8	2.1	2.3	2.3
25	31.3	17.8	12.2	8.0	2.3	2.4	2.2	2.3
30	38.5	25.0	17.2	13.2	2.2	2.0	1.9	1.7
35	71.4	41.7	26.3	18.2	1.8	1.7	1.8	1.8
40	83.3	50.0	33.3	23.0	1.9	1.7	1.6	1.5
45	125.0	71.4	47.6	33.1	1.3	1.3	1.2	0.78
50	161.3	86.2	53.2	34.3	0.56	0.50	0.44	...
55	166.6	96.1	57.5	25.6	...	...	...	...
60	90.9	43.5	23.6	<sup>b</sup>	...	...	...	...

<sup>a</sup> 2 grams keratose per liter; 0.02 gram enzyme No. 6 per liter; pH, 8.0.

<sup>b</sup> Reaction stops.

The temperature coefficient is dependent on the stage of the reaction chosen as the end point in measuring the activity of the enzyme. At lower temperatures, the coefficient found is larger, the nearer to completion the reaction is allowed to proceed. At higher temperatures the temperature coefficient becomes smaller as the reaction progresses. For example, consider the interval 45° to 55° C. For 20 per cent digestion, the time required is 1.3 times as long at 45° as at 55°; for 50 per cent digestion, it takes only 0.78 times as long at the lower temperature.

That the magnitude of the temperature coefficient of the hydrolysis of keratose is dependent upon the nature of the enzyme employed is shown by the results given in Figs. 73 and 74. The rate of digestion of keratose was determined at intervals of 10 degrees with two other samples, in addition to No. 6. One of these samples was a very weak enzyme and the other a very strong one. No. 6 occupies an intermediate position. The temperature coefficient falls off, with increasing temperature, much faster in the case of the strongest enzyme. All these values refer to 40 per cent digestion of the substrate.

In the temperature range available for practical bating, however, neither the purity of the enzyme nor the stage of the reaction has much

effect upon the temperature coefficient. Generally speaking, bating is carried out between 25° and 35° C., and it is safe to say that 40° is the maximum temperature that can be employed for the process, as

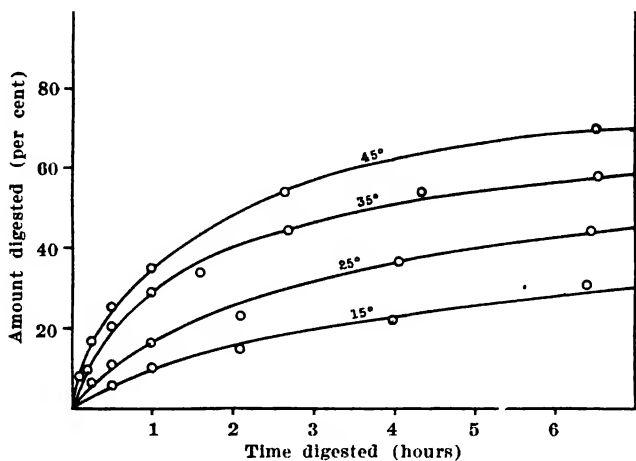


FIG. 73.—Rate of digestion of keratose (keratose, 2 grams per liter; trypsin No. 9, 0.002 gram per liter) at different temperatures.

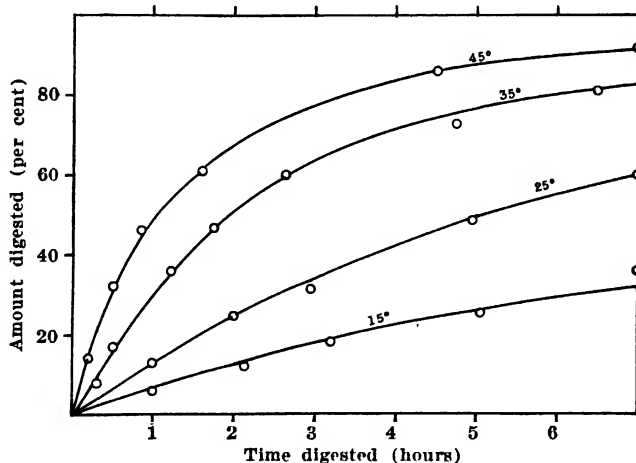


FIG. 74.—Rate of digestion of keratose (keratose, 2 grams per liter; trypsin No. 2, 0.2 gram per liter) at different temperatures.

collagen begins to hydrolyze rapidly a little above this temperature. Confining our attention to the temperature coefficients for the range from 15° to 40°, we see that nearly the same result is obtained whether the fraction of keratose hydrolyzed is 20 or 50 per cent, and whether the enzyme be crude or refined. Throughout this range the tempera-

ture coefficients are in the neighborhood of 2, which means that for a 10-degree rise in temperature a given amount of enzyme will hydrolyze a given amount of keratose in half the time, or half the amount of enzyme will hydrolyze the given amount of keratose in the same time. Thus, so far as the digestion of keratose is concerned, it is possible to bate at any temperature below 40° by making suitable adjustments in the quantity of enzyme used or in the time of bating, or in both.

*Practical Application.* The technic and data on the digestion of keratose by pancreatin, just described, afford a means for determining the amount of any pancreatin sample to use in practice in order to get the desired bating effect. In the bating of calf skins, we have found that an improved leather is obtained up to a certain time of bating, beyond which the leather becomes poorer in quality. For any fixed conditions, different samples of pancreatin have different optimum periods of bating.

As an example, take three different samples of pancreatin, A, B, and C, showing values by the Wilson-Merrill method of 2, 10, and 80, respectively. Using these three samples in bating whole packs of calf skins and varying only the concentration of enzyme, it is found necessary to use 40 times as much of A as of C or 8 times as much of B as of C, in order to get the best effect as determined by the assortment of the leather. Thus a simple laboratory test makes it possible to measure the bating value of any pancreatin sample, as reflected in the quality of the leather produced, and with remarkable precision. This was verified on tests involving tens of thousands of calf skins, proving that the important function of the enzymes in bating calf skins is the digestion of keratose.

### Hydrolysis of Collagen.

The previously widely held view that collagen is not attacked by trypsin was shown to be erroneous by the work of Thomas and Seymour-Jones.<sup>25</sup> Fig. 75 gives their data for the rate of digestion of hide powder by trypsin as a function of time. It is interesting to note also the steady hydrolysis in the blank (without enzyme) at 40° C. In Fig. 76 are shown the rates of digestion of fine and coarse hide powders as functions of the concentration of enzyme. The fine powder consisted of the portion passing through a sieve of 34 meshes to the inch and the coarse powder of the portion retained by the sieve. A much longer time was required to hydrolyze the coarse powder, as expected.

Marriott<sup>7</sup> objected to the finding of Thomas and Seymour-Jones on the ground that the previous treatment of the hide powder with lime or other materials may have so altered the collagen as to make it digestible by trypsin when it may not have been so before. Merrill and Fleming<sup>9</sup> proved that unaltered collagen is attacked by trypsin. They cut away the flesh, epidermis, and hair from fresh calf skin, washed it in pure water, and then subjected it to tryptic digestion. Another sample was limed and then digested with trypsin. They found

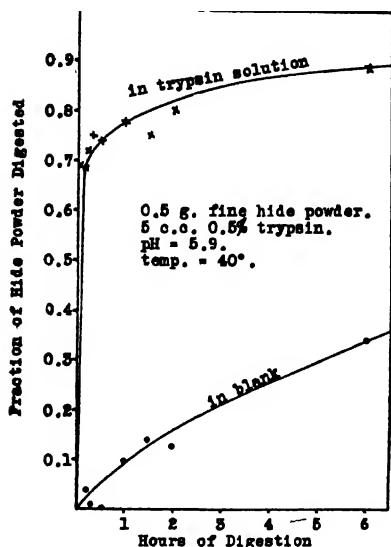


FIG. 75.—Rate of digestion of hide powder by trypsin as a function of time.

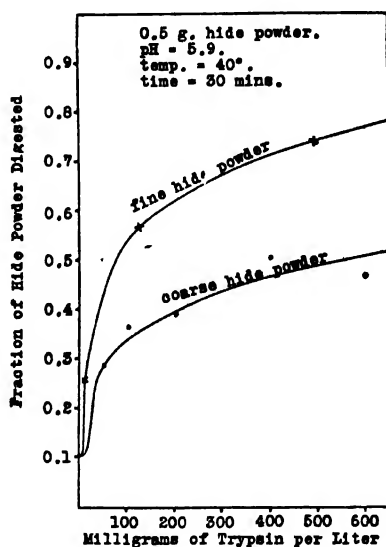


FIG. 76.—Rates of digestion of fine and coarse hide powders as functions of the concentration of trypsin.

that the collagen of fresh calf skin is attacked by trypsin at exactly the same rate as the collagen of limed calf skin, although limed calf skin contains a greater amount of material easily digestible by trypsin, probably consisting of degradation products of a number of proteins present in the skin.

Merrill and Fleming made an interesting study of the nature of the digestion of calf skin by trypsin. They considered three possibilities pictured by the three hypothetical curves in Fig. 77. Curve A represents what would happen if the true collagen of skin were not attacked by trypsin; action would cease after the digestible proteins had been hydrolyzed and the curve would become parallel to the

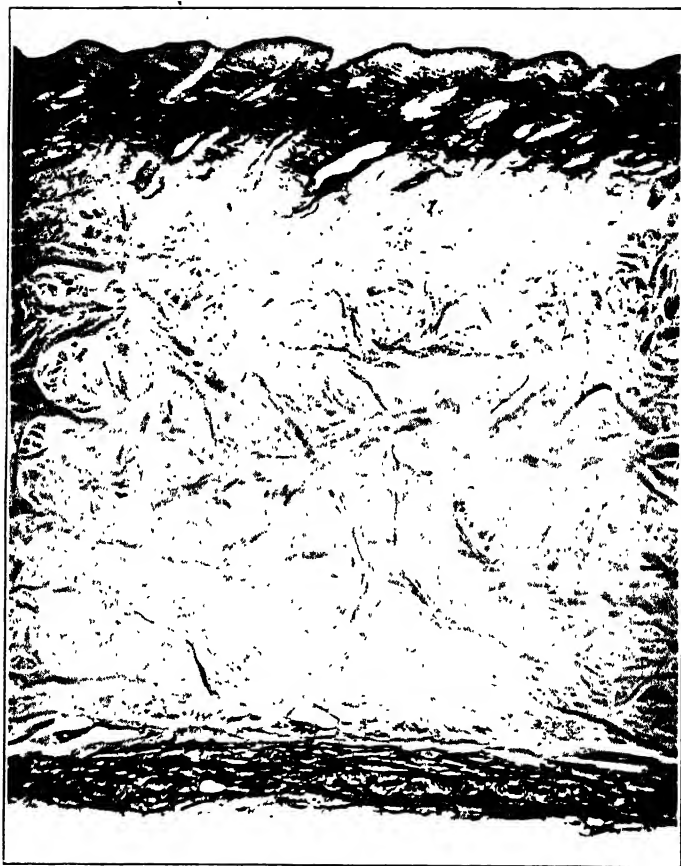




Plate 87.—Bate Master Examining Skins in Bating Paddle Vats.



Plate 88.—Sorting After Bating.



**Plate 89.—Vertical Section of Calf Skin.**  
(After liming and unhairing, before bating.)

Location: butt.

Thickness of section: 40  $\mu$ .

Stains: Weigert's resorcin-fuchsin  
and picro-red.

Eye-piece: 5X.

Objective: 32-mm.

Wratten filters: B-green; E-orange.

Magnification: 35 diameters.



**Plate 90.—Vertical Section of Calf Skin.**  
(After bathing, before tanning.)

Location, butt.

Thickness of section: 40  $\mu$

Stains: Weigert's resorcin-fuchsin  
and picro-red.

Eye-piece: 5X.

Objective: 32-mm.

Wratten filters: B-green; E-orange.

Magnification 35 diameters.



**Plate 91.—Vertical Section of Thermostat Layer of Cow Hide.**  
(After liming and unliming, before bating )

Location butt.  
Thickness of section  $20\ \mu$   
Stain: Daub's bismarck brown

Eye-piece: 5X.  
Objective: 16-mm  
Wratten filter C-blue  
Magnification, 140 diameters.



**Plate 92.—Vertical Section of Thermostat Layer of Calf Skin.**  
(After liming and unliming, before bating)

Location: butt.  
Thickness of section: 20  $\mu$ .  
Stain: Daub's bismarck brown

Eye-piece: 5X.  
Objective: 16-mm.  
Wratten filter: C-blue.  
Magnification: 140 diameters.



Plate 93.—Vertical Section of Thermostat Layer of Sheep Skin.  
(After liming and unhairing, before bathing.)

Location: butt.

Thickness of section: 20  $\mu$ .

Stain: Daub's bismarck brown.

Eye-piece: 5X.

Objective: 16-mm.

Wratten filter: C-blue.

Magnification: 140 diameters.



**Plate 94.—Vertical Section of Thermostat Layer of Hog Skin.**  
(After liming and unhairing, before bating.)

Location: butt.  
Thickness of section: 20  $\mu$ .  
Stain: Daub's bismarck brown.

Eyepiece: 5X.  
Objective: 16-mm.  
Wratten filter: C-blue.  
Magnification: 140 diameters.

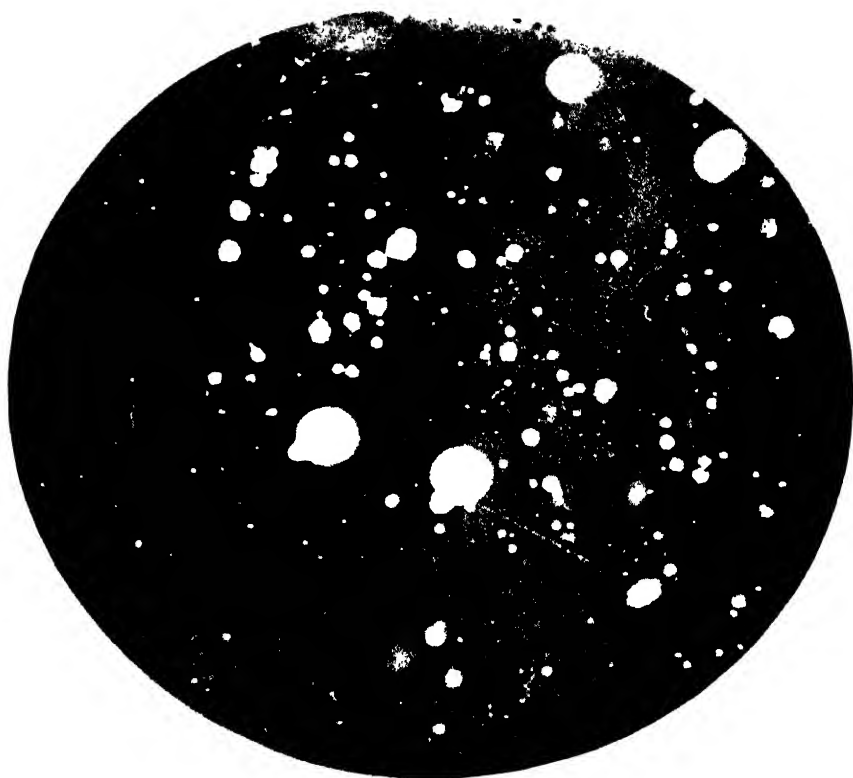


Plate 95.—Typical Plate Culture on Gelatin of Puer Liquor.



axis of time. Curve B shows the behavior of skin on the assumption that the collagen fibers are digestible but are enclosed in a sheath

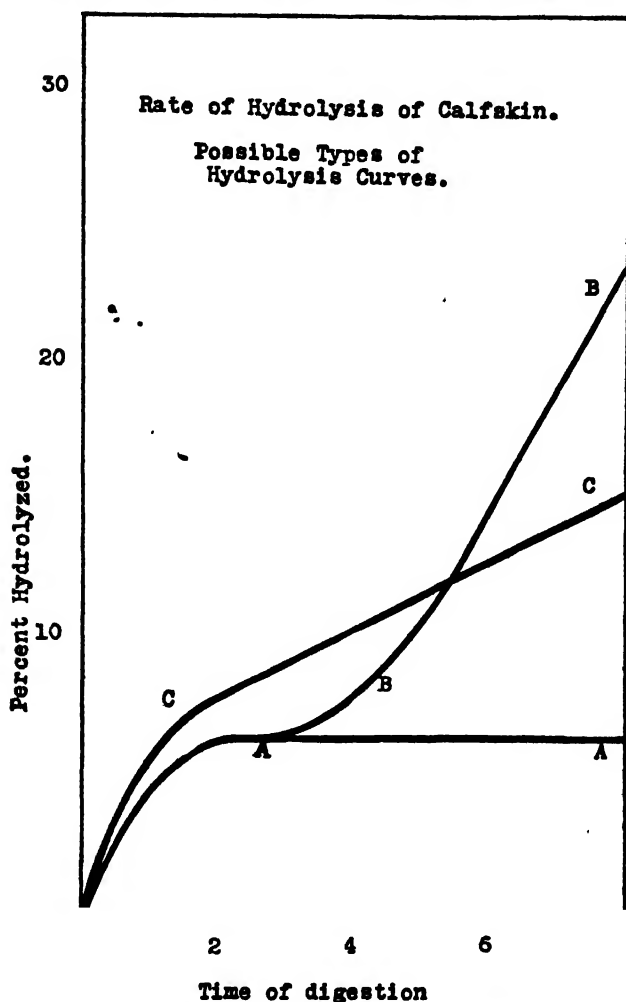


FIG. 77.—Hypothetical curves representing type to be expected if (A) true collagen is not hydrolyzed by trypsin; (B) true collagen is hydrolyzed but protected by sheaths which are attacked only with difficulty; or (C) true collagen is attacked and is not protected by a resistant sheath.

which is attacked by trypsin with difficulty; the curve rises sharply at first, due to the removal of keratose, elastin, etc., then becomes flat until the protecting sheaths are removed, and then shoots upward abruptly as the now unprotected collagen is eaten away. Curve C

represents the behavior of skin when all of the collagen fiber is equally susceptible to the action of the enzyme, with no protecting sheath coming into play. The removal of easily digestible materials causes the curve to rise sharply at first; when these are removed, the curve becomes a straight line, not parallel to the axis of time, but steadily rising as the collagen is hydrolyzed. Their experiments, now to be described, show that this third type of behavior is the one actually manifested by the collagen of calf skin.

In the study of the important variable factors, Merrill and Fleming used calf skin which had been limed, unhaired, scudded, washed, and delimed to the neutral point with HCl. This seemed preferable to using unlimed skin because practical bating is done almost entirely with limed skin. However, later experiments seemed to show that exactly the same results are obtained with fresh collagen. The skin was cut into squares with a 2-mm. side.

The general procedure was to take wet skin containing 1 gram of collagen and digest it for a given time and at a given temperature with 100 cc. of buffer solution of fixed pH value and enzyme concentration. A second sample was treated with exactly the same solution, but in which the enzyme had been inactivated by boiling for 15 minutes. At the end of the first 24-hour period, both solutions were filtered, nitrogen determined in the filtrates, and calculated as collagen. The difference between the two figures was taken as the weight of nitrogenous matter, as collagen, dissolved by the enzyme. Each sample of skin was again treated with a fresh solution, exactly like the first, for a second twenty-four hour period. The nitrogenous matter dissolved in the second day was determined as before, and the whole process repeated until the trend of the hydrolysis curve was unmistakable. The quantities of nitrogenous matter digested in the first, second, third, etc., twenty-four hour periods were added to obtain the total quantity digested in 1, 2, 3, etc., days. The total quantity dissolved was then plotted as a function of time.

For example, with one enzyme preparation, the figures were obtained which are tabulated in Table XXVI.

TABLE XXVI

Period Hours in Period	1st 24	2nd 24	3rd 24	4th 48	5th 24	6th 24
Grams nitrogen (as collagen)						
digested with active enzyme...	0.0590	0.0329	0.0252	0.0409	0.0220	0.0283
With blank .....	0.0157	0.0071	0.0063	0.0118	0.0055	0.0094
By active enzyme .....	0.0433	0.0258	0.0189	0.0291	0.0165	0.0189
Total digested .....	0.0433	0.0691	0.0880	0.1171	0.1336	0.1525

This method has the disadvantage that any error in one day's determination is carried over into all subsequent ones, but as trypsin

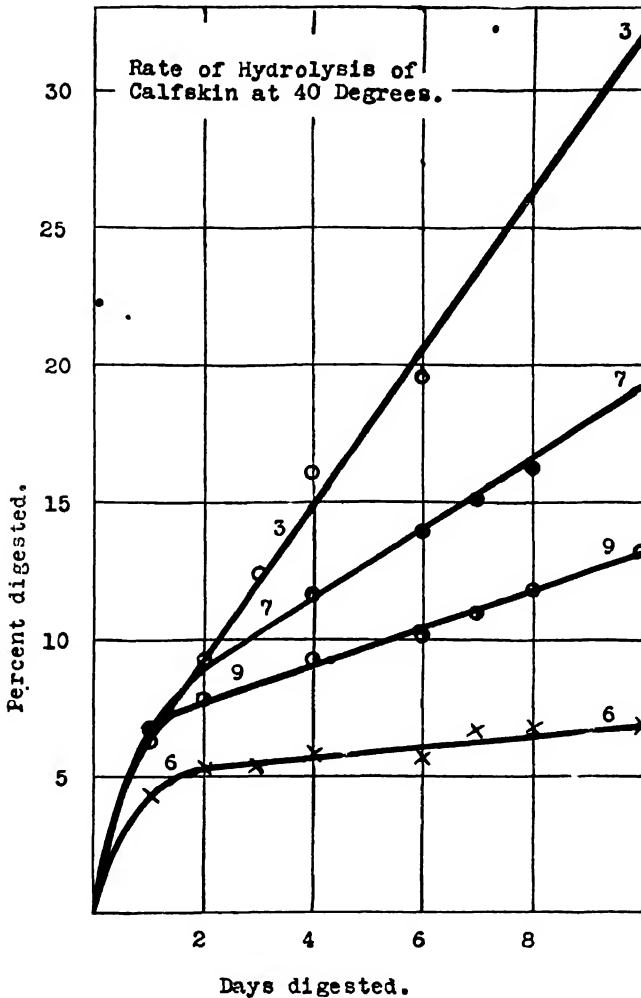


FIG. 78.—Effect of time on the rate of hydrolysis of calf skin by four different samples of trypsin at 40° C.

loses its activity quite rapidly it is necessary to renew the solutions daily in order to measure the digestion over long periods of time.

*Effect of Time.* The effect of time of digestion, over a ten-day period, with four different samples of trypsin is shown in Table XXVII and in Fig. 78. The enzyme numbers refer to the listing in Table

XXIX. Each one of the four curves conforms to type C in Fig. 77. In each case the curve rises steeply during the first day and then bends off, becoming a straight line, but of different slope for each different enzyme. The rapid rise during the first day is due to the hydrolysis of the easily digested materials, such as keratose and elastin. The linear relationship between amount of collagen digested and the time, after the first day, points to the hydrolysis of collagen. Indeed, the digestion of collagen is proved by the very magnitude of the quantities of nitrogenous matter removed. In one case nearly 30 per cent of the total skin had passed into solution in ten days, and still there was no sign of diminishing rate of hydrolysis. The difference in the slopes of the curves indicates the difference in the activities of the different enzymes on collagen.

TABLE XXVII

RATE OF DIGESTION OF CALF SKIN BY TRYPSIN.

5.0 grams delimed calfskin (= 1.0 gm. dry wgt.)

100 cc. solution

pH = 8.0

T = 40° C.

Enzyme No.	Grams Per Liter	Activity on Hide Powder	Grams Nitrogen (as Collagen) Dissolved in — Days							
			1	2	3	4	6	7	8	10
3	0.75	1.8	0.0614	0.0921	0.1244	0.1606	0.1944	—	0.2274	—
6	0.10	13.3	0.0433	0.0538	0.0546	0.0577	0.0562	0.0664	0.0672	0.0672
7	0.05	31.3	0.0669	0.0890	0.1024	0.1260	0.1394	0.1505	0.1622	0.1780
9	0.03	38.5	0.0669	0.0779	0.0834	0.0928	0.1007	0.1101	0.1187	0.1313

*Effect of pH Value.* Fig. 79 shows the effect of pH value on the action of 0.1 gram per liter of enzyme no. 6 for 24 hours on calf skin at 35° and at 40° C. and on hide powder at 40°. It should be noted that the scale for hide powder is ten times as great as for calf skin.

The maximum rate of digestion of calf skin occurs at about pH = 8, both at 35° and at 40°. With hide powder, the maximum occurs at a higher pH value. It is possible that the value obtained for skin is influenced by the presence of materials other than collagen in the skin and that the actual maximum for collagen occurs at a pH value greater than 8, as indicated by hide powder.

*Effect of Surface Exposed.* It will be noted in Fig. 79 that about 12 times as much hide powder as calf skin was digested in 24 hours. Finely divided hide powder is hydrolyzed more rapidly than coarser powder, as shown in Fig. 76. Where it takes ten days to hydrolyze only 40 per cent of a given sample of calf skin, it takes only a few hours to hydrolyze a corresponding sample of hide powder completely.

The reason for this difference undoubtedly lies in the surface area of collagen exposed to the action of the enzyme. This is indicated both by the work of Thomas and Seymour-Jones and some unpublished work by Merrill. Collagen is insoluble and, hence, the action can proceed only at the surface exposed to the action of the solution. In order to illustrate this, Merrill took two samples of purified and screened hide powder, one of 60 to 80 mesh and the other 20 to 40

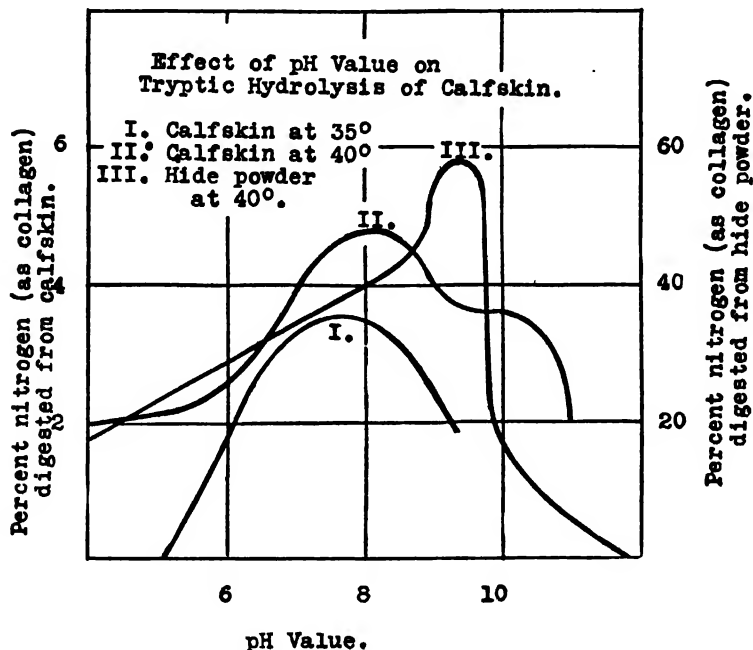


FIG. 79.—Effect of pH value on the hydrolysis of calf skin and of hide powder by 0.01 gram enzyme No. 6 in 100 cc. acting upon 1 gram collagen for 24 hours at indicated temperature.

mesh. In each test, powder equal to 0.500 gram collagen was digested in 100 cc. of solution of enzyme of  $\text{pH} = 7.9$  for 3 hours at 40° C.

The results are shown in Fig. 80. The more rapid digestion of the finer powder is clearly shown, but it is also shown that the addition of enzyme beyond a given concentration has no additional effect. The reason for this is that a maximum rate of digestion is reached when the surface of the collagen is completely saturated with the enzyme. An increase in rate will, however, follow an increase in degree of subdivision of the collagen with corresponding increase in surface area exposed.

*Effect of Temperature.* Fig. 81 gives Merrill's results<sup>8</sup> on the effect of temperature on the rate of hydrolysis of hide powder by trypsin. In each test, 0.5 gram of collagen was digested for 3 hours at the given temperature with 100 cc. of solution of pH = 8, containing the indicated amount of enzyme. For each 10° rise of temperature, there is a relatively enormous increase in rate of hydrolysis.

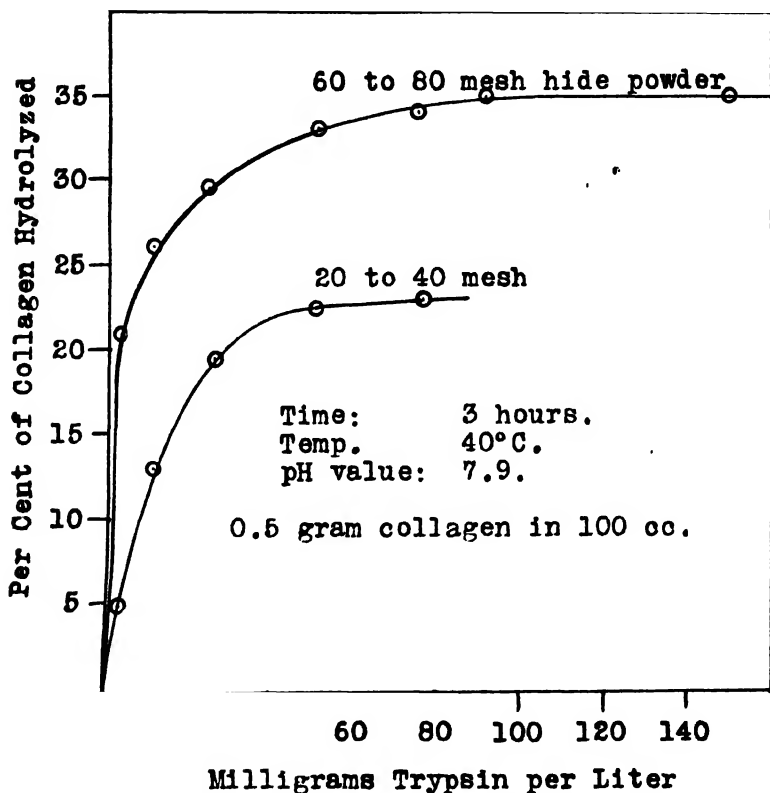


FIG. 80.—Rate of hydrolysis of hide powder as a function of degree of subdivision and of concentration of trypsin.

On comparing the curves of Fig. 78 with those of Fig. 82, which are drawn to the same scale, it is evident that the hydrolysis is enormously greater at 40° than at 35°. In fact, the actions of enzymes numbered 6, 7, and 9 at 35° become almost immeasurable after the first day. The actual values are given in Table XXVIII for comparison with the values in Table XXVII. It is worthy of note that the quantity of material removed the first day, before the curves

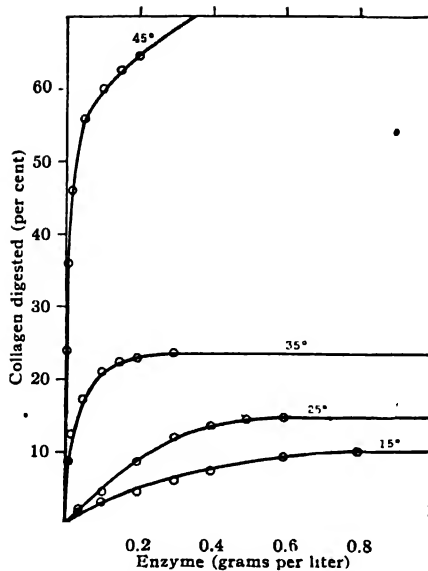


FIG. 81.—Rates of hydrolysis of hide powder by enzyme at different temperatures.

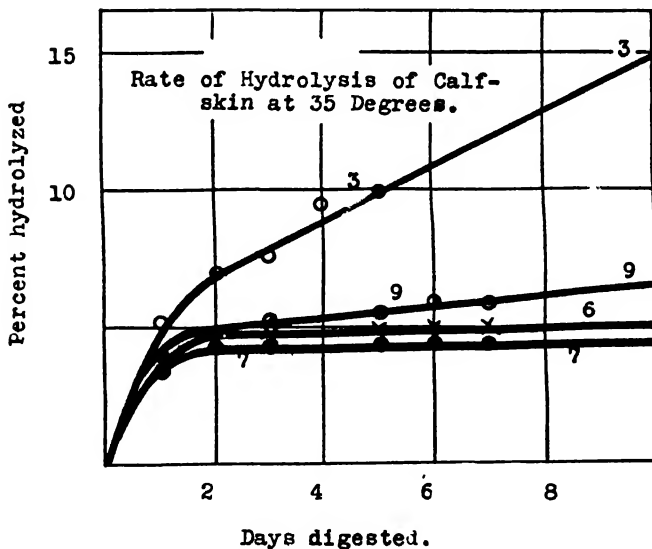


FIG. 82.—Rates of hydrolysis of calf skin with different enzyme samples at 35° C.

become straight, is practically the same at both temperatures, indicating that the quantity of enzyme taken was enough to digest all of the easily hydrolyzable materials, even at 35°.

Where the temperature of bating is kept below 35° C. and only enough enzyme is used to digest the keratose or elastin in the course of a few hours or a day at most, it is apparent that not more than a negligible amount of collagen will be dissolved by the enzyme.

TABLE XXVIII

RATE OF DIGESTION OF CALF SKIN BY TRYPSIN.

5.0 grams delimed calfskin (= 1.0 gm. dry wgt.)

100 cc. solution.

pH = 8.0.

T = 35° C.

Enzyme No.	Grams Per Liter	Activity on Hide Powder	Grams Nitrogen (as Collagen) Digested in — Days						
			1	2	3	4	5	6	7
3	0.75	1.8	0.0512	0.0693	0.0756	0.0944	0.0999	—	—
6	0.10	13.3	0.0363	0.0473	0.0473	—	0.0473	0.0495	0.0503
7	0.05	31.3	0.0346	0.0425	0.0425	—	0.0448	0.0448	0.0448
9	0.03	38.5	0.0394	0.0479	0.0527	—	0.0550	0.0582	0.0582

*Effect of Neutral Salts.* The peculiar action of certain neutral salts upon hide substance was described in Chapter 7. Chlorides, for example, seem to form addition compounds with those groups in the protein molecule which are held together by secondary valency forces and to separate them. The effect on collagen seems to be to carry it into solution as gelatin.

Stiasny and Ackermann<sup>22</sup> studied the action of trypsin on hide powder swollen with different neutral salts at pH = 8.6. The swelling followed the order  $\text{CNS} > \text{I} > \text{NO}_3 > \text{ClO}_3 > \text{Cl} > \text{SO}_4$ . The rate of *solution* of the hide powder by trypsin (as shown by decrease in volume in a centrifuge tube) was greatest in the presence of CNS and at the concentration of this ion producing the maximum swelling. The rate of *solution* of hide powder fell off in the same order as the swelling, but the *hydrolysis*, as measured by decrease in matter precipitable by tannin or the increase in formol titration was not affected by the salts, except that in very high concentration of salt, the trypsin was inactivated. This shows that swelling by salts promotes the initial deaggregation of the collagen, but not the more profound hydrolysis by the enzyme. Stiasny and Das Gupta<sup>23</sup> found that the salts did not increase the hydrolysis of gelatin in solution by enzymes.

### Evaluation of Bating Materials.

Although it has long been known that enzymes are extremely selective in their actions, it has become customary to measure the proteolytic activity of a given enzyme preparation by its action upon



casein, even though it is to be used in the hydrolysis of some other protein. This practice is extremely misleading in the examination of enzymes for bating, as shown by the following comparison of two samples of pancreatic enzymes A and B. In the hydrolysis of casein, A was found to be 48 times as powerful as B, but in the digestion of the skin protein elastin B was found to be 100 times as powerful as A. If B had been purchased to replace A in the digestion of elastin, the casein test would have called for the use of 4800 times as much as actually required.

Pancreatin, the enzyme preparation used in bating, contains three classes of enzymes: (1) amylases or diastases which convert starches into sugars; (2) lipases which hydrolyze fats; and (3) proteases which hydrolyze proteins. The pancreatic proteases are often referred to as trypsin, as though they were a single enzyme instead of a mixture of proteolytic enzymes none of which has ever been isolated in a pure state. The various enzymes in commercial pancreatins are present in such varied proportions that a comparison of activities of several samples upon one protein gives no reliable clew to their comparative activities upon some other protein.

In bating, any or all of the skin components may be attacked by enzymes, so it is unsafe to use any new enzyme preparation in bating until it is known just how it will act upon each important constituent of the skin. Even highly purified pancreatins may contain the different enzymes in very different proportions according to the method of preparation.

Since no enzyme has yet been prepared in a pure state, the only means at our command for evaluating an enzyme preparation is to measure the amount of substrate which a given amount of the sample will hydrolyze under rigidly controlled conditions. By substrate is meant the material upon which the enzyme acts; for example, casein is the substrate when this protein is being hydrolyzed by an enzyme. To facilitate the comparison of different samples, purely arbitrary units of strength are set up for each class of enzymes.

In measuring the amount of work performed by an enzyme, we may start with a definite amount of substrate and a definite amount of enzyme and allow the reaction to proceed for a definite period of time. We may then determine the quantity of unchanged substrate or the quantity of products formed, either directly or by measuring some physical property that changes as a function of the progress of the reaction. Thus, if we are measuring the activity of a tryptic enzyme on casein, we can measure the amount of casein still unchanged

by precipitating it at its isoelectric point, filtering, and weighing it. Or we can measure the amount of nitrogen in the filtrate, thus getting the amount of reaction product formed. If gelatin is used as substrate, the reaction may be followed by measuring the changing viscosity of the solution.

Where possible, it is preferable to determine the amount of unchanged material, since very often the first products of the reaction undergo further changes. In interpreting the data thus obtained so as to evaluate and compare different samples, three schemes have been employed:

- (1) Measurement of the amount of substrate acted upon in a definite time with fixed initial quantities of substrate and of the enzymes being compared.

- (2) Measurement of the time required for a fixed quantity of enzyme to act on a definite amount of substrate.

- (3) Measurement of the amount of enzyme required to hydrolyze a definite amount of substrate in a given period of time.

The first of these procedures is objectionable because the rate of reaction depends upon the concentration of the unchanged material, which is itself a variable. With a very active enzyme the reaction will proceed very rapidly at first and then will slow down much more rapidly than is the case with a less active enzyme material when using the same weight of both materials. The amounts of material acted upon by the two enzymes will thus tend to approach each other, the longer the time period employed. This is aggravated by the fact that the enzyme itself is frequently destroyed during the reaction and the destruction increases with the amount of reaction products formed.

While the second and third procedures are equally sound in theory, the second is less convenient if samples of very different activity are to be compared. The third is generally the best and is most frequently employed. Instead of measuring the amount of enzyme required to cause complete hydrolysis of the substrate in a given time, as is often done, it is better to measure the quantity required to act on a definite fraction of the substrate, say 40 per cent. During the first part of the reaction, the classical laws of chemical kinetics are followed with fair accuracy, but during the later stages the operation of these laws is masked by many disturbing factors, such as the combination of the enzyme with the reaction products, successive reactions, reverse actions, etc.

The activity of the enzyme is inversely proportional to the time required to bring about the decomposition of a definite amount of

substrate or to the quantity of enzyme required to bring about the specified change in definite time. In the methods developed by Wilson and Merrill<sup>81</sup> for estimating the activity of enzymes on keratose and other soluble protein materials, they have expressed activities in terms of the fraction  $1/hg$ , where  $h$  is the number of hours required for the specified change and  $g$  is the number of grams of the enzyme sample per liter. The value of  $g$  is made great enough to bring about the specified change in reasonable time and  $h$  is the independent variable. Difficulties involved in working with the insoluble substrates collagen and elastin have made it more suitable to fix  $h$  arbitrarily and to make  $g$  the independent variable, the activity then being measured by the fraction  $1/g$ .

Lipolytic activity is measured by the amount of enzyme required to hydrolyze a given amount of oil in a given time.

It is extremely important to maintain constant temperature and pH value during the course of these reactions and so they selected a temperature of 40° and a pH value of 7.9, this being in the range of greatest activity for the pancreatic enzymes and also in the range found in practical bating. Control of pH value is effected by the use of buffer salts. With insoluble substrates requiring agitation, it is essential that the amount of agitation be made the same in all determinations. It is also necessary to preclude bacterial action by the use of suitable antiseptics. Some substrates may be hydrolyzed to some extent in the absence of the enzyme; in such cases, blanks without enzyme must be run and corrections made accordingly on the tests with enzyme.

#### Activity on Keratose (Wilson-Merrill Method).

The activity of an enzyme sample on keratose is defined as the reciprocal of the product of the concentration of enzyme in grams per liter and the number of hours required to digest 40 per cent of the keratose present in the solution to the extent of 2 grams per liter at 40° C. and  $\text{pH} = 7.9$ .

*Preparation of keratose solution:* Digest 100 grams of washed white calf hair with 1000 cc. of 2-normal NaOH at 25° C. for 18 hours. Then add HCl slowly until the pH value has been reduced to about 8; the end-point can be ascertained closely enough by spotting on a tile with phenol red and thymol blue indicators. Filter through folded Swedish filter paper.

Add dilute HCl to the filtrate, with stirring, until a copious, permanent, white precipitate forms, but be careful not to add more than

enough acid to get this precipitate. Then add 200 cc. of Buffer Solution No. 2 (see below). Stir thoroughly and pour into a tall cylinder. Allow the precipitate to settle and test the supernatant liquid for complete precipitation by adding more of Buffer Solution No. 2. Continue to add the buffer until precipitation is complete. Keep the mixture cold, preferably in a refrigerator. Allow the precipitate to settle and wash three times by decantation. \*

Suspend the precipitate in about 200 cc. water and add normal NaOH gradually and with stirring until the pH value of the solution is 8.0, as shown by spotting with the indicators. Keep stirring and add more NaOH if necessary to maintain a pH value of 8.0 until all of the keratose has passed into solution. Then transfer to a stoppered bottle, add a crystal of thymol, and keep in the refrigerator until required for use.

*Buffer solution no. 1:* Make up to 2,000 cc. an aqueous solution containing 61.7 grams of disodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ), 60.7 grams of sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 5.5\text{H}_2\text{O}$ ), 10.3 grams boric acid and enough NaOH or HCl to make a final pH value of 8.0. One hundred cc. of this solution in a liter of digestion mixture will maintain practical constancy of pH value during the digestion.

*Buffer solution no. 2:* Make up to 2,000 cc. 60 grams of acetic acid and enough NaOH to give a final pH value of 3.6. When 50 cc. of this solution is added to 100 cc. of digestion mixture containing 10 cc. of Buffer Solution No. 1 and 0.2 gram keratose, the pH value is brought to 4.1, the isoelectric point of keratose and the point at which it is precipitated.

*Digestion mixture:* Determine the concentration of keratose in the stock solution as follows: Pipette 10 cc. of the stock solution into 80 cc. of water and 10 cc. of Buffer Solution No. 1. Add 50 cc. of Buffer Solution No. 2 and allow the precipitate which forms to settle for 30 minutes and then filter through a Whatman No. 40 filter paper which has previously been dried at  $100^\circ\text{C}$ ., cooled in a desiccator and weighed. Wash the precipitate four times with very dilute HCl (0.00025 normal). Dry the filter paper and residue in the oven at  $100^\circ\text{C}$ . over night, transfer to a stoppered weighing bottle, cool in a desiccator, and weigh.

Pipette a volume of the keratose solution containing exactly 2 grams of keratose into a liter flask, add 100 cc. of Buffer Solution No. 1, dilute to about 950 cc., and warm to  $40^\circ\text{C}$ . Weigh off a quantity of the enzyme sample sufficient to digest 40 per cent of the keratose, that is, 0.8 gram, in from 1 to 3 hours. If the first guess as

to quantity to take is far out, the test should be repeated with a proper amount. As a first approximation, one may take 1 gram of ordinary commercial bating materials, 0.1 gram of U.S.P. pancreatin, or 0.01 gram of highly purified enzyme. Dissolve the enzyme in 25 cc. of water at 40° C., add to the keratose solution in the flask, and make up to exactly 1,000 cc. Keep the flask in a thermostat at 40° and count time from the moment of adding the enzyme.

Withdraw aliquots of 100 cc. each from the flask after the lapse of definite periods of time, say 0.25, 0.5, 1.0, 1.5, 2, 3, and 4 hours. Immediately upon the withdrawal of an aliquot, add to it 50 cc. of Buffer Solution No. 2, allow the precipitate to settle for 30 minutes, filter through a tared paper, wash with dilute HCl, dry, and weigh, as described above. This gives the weight of the undigested fraction of the keratose plus a small amount of insoluble matter from the enzyme.

*Corrections:* To correct for the insoluble material in the enzyme, make up a second liter of enzyme solution as described above, but leave out the keratose. Withdraw 100 cc. and treat exactly as for the 100-cc. aliquots from the digestion mixture. Deduct the weight of residue found from the weight of each residue from the digestion mixture. Often this deduction is so small that it can be ignored. As long as the time of digestion is kept within three or four hours, it is not necessary to make any correction for keratose digested in the absence of enzyme.

*Calculation of activity:* The amount of keratose digested at the end of any given time period, per 100 cc., is the difference between the weight originally present and that found in the residue after the given digestion period, a correction being made where necessary for the insoluble matter introduced in the enzyme. The per cent of the total keratose digested is then plotted as a function of time. For example, with one sample of a commercial pancreatin, the following results were obtained:

Time of Digestion (hours)	Grams of Residue (corrected)	Percent Digested
initial	0.2020	none
0.25	0.1807	10.54
0.50	0.1625	19.55
0.90	0.1350	33.22
1.50	0.1114	45.40
2.95	0.0775	61.98

These figures are plotted in Fig. 83. The next step is to find the number of hours required to digest 40 per cent of the keratose. The 40 per cent line is drawn to the point where it intersects the smooth

curve drawn through the plotted points and from the point of intersection a perpendicular is dropped to the horizontal axis, indicated in Fig. 83 by dotted lines. The perpendicular corresponds to a time period of 1.2 hours. In this digestion, we took 0.2 gram of the enzyme

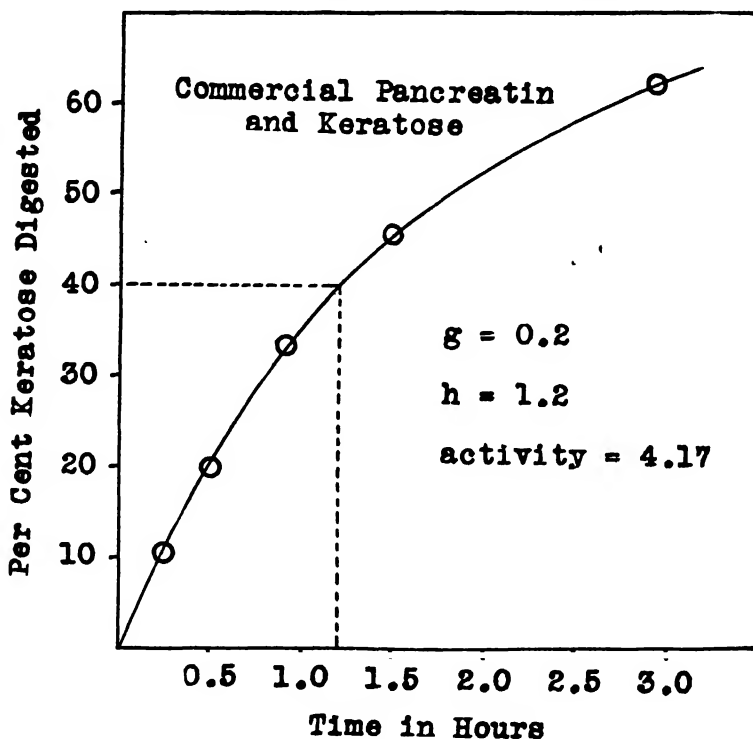


FIG. 83.—Digestion of keratose by a commercial pancreatin as a function of time. Chart showing method employed in measuring the activity of the enzyme on keratose.

per liter. Therefore  $g = 0.2$  and  $h = 1.2$  and the activity of the enzyme on keratose  $= 1/hg = 4.17$ .

The amount of enzyme and the time periods should be so chosen that at least two points are obtained on each side of the 40 per cent digestion point.

#### Activity on Elastin (Wilson-Daub Method).

The activity of an enzyme sample on elastin fibers is defined as the reciprocal of the number of grams of enzyme per liter required to remove all of the elastin fibers from limed calf skin in 24 hours at 40° C. and pH = 7.9.

As a buffer solution a large reservoir of sodium phosphate is kept on hand whose composition is equal to that of a 0.02-molar solution of phosphoric acid with enough added NaOH to make the pH value 7.9. About 10 bottles of this solution are taken, each holding about 500 cc. These form a series in which increasing amounts of the enzyme sample are dissolved. The temperature is raised to 40° C. and into each is placed a strip of limed, unhaired and washed calf skin about 2.0 x 0.5 inch. The bottles are kept in a thermostat at 40° for 24 hours, with occasional shaking. At the end of this time, the strips from all bottles, suitably marked, are washed in running tap water for 1 hour and then put into 80 per cent alcohol. After 1 day they are transferred to 95 per cent alcohol and after another day to absolute alcohol. After about 12 hours this is replaced by fresh absolute alcohol and after another half day this is replaced by a mixture of equal volumes of absolute alcohol and xylene. This in turn is replaced after 12 hours by a mixture of 1 volume of melted phenol and 3 volumes of xylene and this, after another 12 hours, by pure xylene. After 12 hours this is replaced by fresh xylene and after another half day the strips are soaked in five changes of molten paraffin about an hour apart. They are then imbedded in paraffin blocks and put into the microtome for sectioning.

Sections are then cut at 30 microns, fixed to microscope slides, rinsed in turn with xylene, absolute alcohol, and 95 per cent alcohol, and then left for 2 hours in Weigert's stain diluted 1 to 4. Then the slide is rinsed in turn with 95 per cent alcohol, phenol-xylene, and xylene. A drop of Canada balsam is placed on the section and it is then covered with a cover glass and is ready for examination under the microscope. The elastin fibers where present are stained a deep blue which makes it easy to follow their removal by the enzyme.

The strength of the enzyme is calculated from the value of the lowest concentration of enzyme which has removed all of the elastin from the skin in 24 hours. It may happen that the concentration intervals were taken too far apart to permit an accurate measure of activity. For example, suppose that 0.4 gram per liter of enzyme removed all of the elastin, but that the next weaker enzyme solution tested contained only 0.1 gram per liter. The activity is measured by the fraction 1/g. In this case it would be possible to say only that the activity lies between 2.5 and 10.0. If it were desired to get the activity accurate to one unit, it would be necessary to start a second series with the following concentrations, in grams per liter: 0.111, 0.125, 0.143, 0.167, 0.200, 0.250, and 0.333. In order to get the activity

of any enzyme accurate to a single unit, it will rarely require more than two series of ten each.

### Activity on Collagen (Wilson-Merrill Method).

The activity of an enzyme on collagen is defined as the reciprocal of the number of grams per liter of the enzyme required to digest 20 per cent of 5 grams of purified hide powder suspended in a liter of solution at 40° C. and pH = 7.9 in 3 hours.

*Purification of hide powder:* The hide powder for this determination is prepared from standard hide powder as follows: Place 1 lb. of hide powder in a jar and cover with 7 liters of distilled water. Agitate occasionally for half a day and then pour the mixture into a large cheese cloth and squeeze out as much water as possible. Return the powder to the jar and repeat the operation until the powder has had five changes of water. It is then treated in a similar manner twice with 40 fl. oz. of 95 per cent alcohol, twice with 40 fl. oz. of a mixture of equal parts of 95 per cent alcohol and xylene, and once with 40 fl. oz. of pure xylene. After the xylene wash, the powder is allowed to remain in a current of warm air until free from the odor of xylene. The product is free from easily soluble nitrogenous matter and from fat. About 15 oz. of the purified product can be obtained from a pound of the original hide powder.

With insoluble protein fibers, like hide powder, the rate of hydrolysis by enzymes is influenced by the specific surface exposed. In order to keep this as uniform as possible, the hide powder is sifted through screens, and that portion only is used which passes a 20-mesh screen, but is retained by a 40-mesh screen.

The hide powder is kept in a stoppered bottle to prevent wide changes in moisture content. It is analyzed for water and for collagen, which is taken as 5.62 times the nitrogen content. In all experiments an amount of hide powder representing exactly 0.500 grams of collagen was used with 100 cc. of digestion mixture.

*Procedure:* Into each of 15 stoppered bottles put hide powder containing exactly 0.500 grams of collagen. Add to each 10 cc. of Buffer Solution No. 1 (see under keratose) and 40 cc. of water, and a crystal of thymol or a drop of toluol to prevent bacterial action. Keep the bottles in a thermostat at 40° C. for one hour. Take bottle No. 15, add to it 50 cc. water, mix, and filter. Determine the amount of nitrogen in the filtrate, and calculate as the amount of collagen dissolved during the preliminary soaking. In the example to be cited this



filtrate contained nitrogen equivalent to 10 mg. of collagen, from which it was concluded that each bottle contained  $500 - 10 = 490$  mgm. collagen at the end of the preliminary soaking.

Two 0.1-gram portions of enzyme are dissolved in a small quantity of water. One portion, to be used for the blanks, is inactivated by boiling for fifteen minutes. Each portion is then made up to exactly 100 cc. Into bottles No. 1-7, immediately after the preliminary soaking of one hour, are placed increasing amounts of the active enzyme solution, and water to make the total volume 100 cc. Into bottles No. 8-14 are placed the same respective quantities of the inactivated enzyme solution and water. The quantities of enzyme taken are varied so as to cover a considerable concentration range. In the example to be cited, the following quantities of enzyme were added to bottles 1 to 7: 0.5, 1, 3, 5, 7, 10, and 20 milligrams. If the results of the first series indicate that the enzyme concentrations used did not cover the critical range sufficiently well, a second series may be run.

At the moment of mixing the enzyme solution and the water with the liquor containing the hide powder, all three components must be at  $40^{\circ}$  C., and the bottles must be kept in a thermostat at  $40^{\circ}$  C. during the digestion. Start counting time from the moment the enzyme was added, and remove all the bottles after exactly three hours. Upon removing each bottle, start filtering immediately through a dry filter, returning the filtrate until it comes through practically clear. Determine the amount of nitrogen in the filtrate, and calculate as collagen ( $N \times 5.62$ ).

Now plot the results as a function of concentration of enzyme. This is illustrated for a special test in Fig. 84. The top curve gives the number of milligrams of nitrogen as collagen found in the filtrates from the active enzyme solutions and the crosses that found in the filtrates from the inactivated enzyme mixtures. The amount of collagen digested by the enzyme itself is represented by the differences between these two curves, shown by the heavy continuous line. It should be noted that subtraction of the blank values from the digestion mixture values makes the necessary correction for the nitrogen introduced with the enzyme as well as that digested in the preliminary soaking period of one hour.

The activity of the enzyme on collagen is obtained from the corrected enzyme curve. The initial amount of collagen present upon adding the enzyme was 490 milligrams, and 20 per cent of this is

98 milligrams. By drawing lines as shown in the figure, it will be seen that a digestion of 98 milligrams occurs at an enzyme concentration of 0.033 grams per liter. The activity then equals  $1/g = 1/0.033 = 30$ .

There is a danger in assuming that values by this method may be applied directly to the bating of skins. In order to attack the collagen

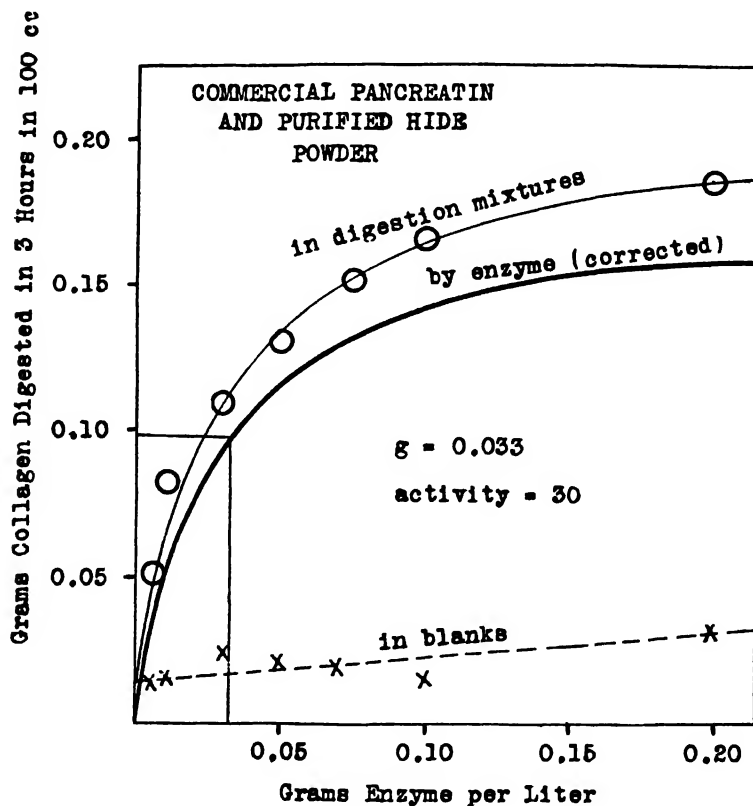


FIG. 84.—Digestion of purified hide powder by a commercial pancreatin as a function of concentration of enzyme. Chart showing method employed in measuring the activity of the enzyme on collagen.

fibers in the interior of a skin, the enzyme must diffuse into it. But the rate of diffusion of an enzyme may be influenced by the nature of the material associated with it. An enzyme might have a high activity on collagen, but a low power of diffusion, and thus produce less effect upon skin than a weaker enzyme of higher diffusive power. This may explain the results in Table XXVII, where the order of activity on hide powder is not the same as on calf skin.

### Activity on Casein.

The only reason for including the methods for determining the casein digesting power of enzymes is that pancreatic enzymes are often sold on the basis of their proteolytic activity as measured by their action on casein. As a matter of fact the power of a commercial pancreatin to digest casein bears no relation to its power to digest keratose, elastin, or collagen.

One of the most widely used methods for measuring casein digesting power is the well known Fuld-Gross method, described in many books on physiological chemistry. This method measures the amount of enzyme required to digest all of a definite quantity of casein in an hour. Results are expressed in Fuld-Gross units in which a value of 100 indicates that 1 milligram of the enzyme can digest 100 milligrams of casein in just one hour.

*Fuld-Gross Method.* Dissolve 100 milligrams of casein, prepared according to Hammarsten, in 2 cc. of 0.05-normal NaOH with the aid of gentle heating and dilute to 50 cc. with water. Dissolve 100 milligrams of the enzyme in water and dilute to 500 cc. Pipette 5 cc. of casein solution into each of ten test tubes. To successive tubes add the following volumes of enzyme solution and then water from a burette to make a final volume of 10 cc.: zero, 0.2, 0.4, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, and 5.0 cc. Shake and keep in the thermostat at 40° C. for one hour.

Make up a solution of 50 cc. alcohol, 5 cc. glacial acetic acid, and 45 cc. water. After the tubes have been digesting exactly one hour, add three drops of this acid mixture to each. The formation of a precipitate indicates the presence of some casein still undigested. If all tubes give a precipitate, the test must be repeated using higher concentrations of enzyme; if no tube gives a precipitate, the test must be repeated using lower concentrations of enzyme.

The activity of the enzyme is measured by the lowest concentration which will digest all of the casein in the hour. Thus, in a given test, upon the addition of the acid mixture, precipitates formed in tubes 1, 2, and 3, but not in the others. Tube 4 thus represented the lowest concentration of enzyme which would digest all of the casein in an hour and it contained 0.7 cc. of the enzyme solution or 0.14 milligrams. The activity in Fuld-Gross units equals the ratio of casein to enzyme or  $10/0.14 = 71$ . A closer approximation to the true value is then obtained by running a second series of ten with closer concentration intervals ranging from that of tube 3 to that of tube 4.

The Fuld-Gross method has two outstanding points of weakness. The most serious weakness is that it does not provide for control of pH value during the digestion. Another weakness is that its end-point is that of total digestion of the casein whereas it is preferable to take as end-point some fraction of the total digestion, say 40 per cent.

*Northrop Method.* A more satisfactory method is based upon a procedure described by Northrop.<sup>18</sup> It is almost exactly the same as the procedure described above for keratose, but with the following changes. The stock solution is prepared by dissolving several grams of pure casein in 100 cc. of Buffer Solution No. 1 and 900 cc. of water. Undigested casein is precipitated by the addition of a sodium acetate-acetic acid buffer solution of  $\text{pH} = 4.1$  so as to make a final pH value of 4.7, the isoelectric point of casein. Washing is carried out with 0.00015-normal HCl. The activity is expressed as the value  $1/hg$ , exactly as for keratose.

#### Activity on Fats.

Apparently no critical work has been done on the action of pancreatic enzymes upon the fatty constituents of skin in bating. However, pancreatin contains lipases, the fat-splitting enzymes, and any measurement of the enzyme activity of a bating material would not be complete without a determination of its fat-splitting or lipolytic activity. Pending the working out of a method involving the use of natural skin greases, methods are being used in which olive oil is the substrate. Two of these methods will be described. It must be borne in mind that the relative activities of two enzymes towards olive oil may be very different from their relative activities towards the skin greases.

*Willstaetter-Waldschmidt-Leitz-Memmen Method.*<sup>26</sup> The lipolytic activity of the enzyme sample is defined as the reciprocal of the number of grams of the sample required to hydrolyze 24 per cent of 2.5 grams of pure olive oil in 1 hour under the specific conditions prescribed below.

Into each of 7 small, glass-stoppered flasks weigh exactly 2.50 grams of pure olive oil (saponification value = 185.5) and add 12 cc. of the following buffer solution: 670 cc. N/10  $\text{NH}_4\text{Cl}$ , 330 cc. N/10  $\text{NH}_4\text{OH}$ , and 200 cc. water. In succession, add to the 7 flasks the following amounts of the enzyme: none, 0.01, 0.02, 0.05, 0.10, 0.15, and 0.20 gram. The flask with no enzyme serves as the blank. Immediately after adding the enzyme, shake by hand for 3 minutes

and then keep in the thermostat at 40° C. for exactly 57 minutes, making a total time of contact of oil and enzyme of one hour.

At the end of the hour, pour the contents of each flask into a titrating vessel, rinsing out the flask with 50 cc. of a mixture of 5 parts of neutral alcohol and 1 part of neutral ethyl ether. Add 10 drops of 1 per cent alcoholic phenolphthalein and titrate with N/10NaOH to the first permanent pink color.

In this titration, alkali is consumed by the following:

1. Free fatty acid liberated by enzyme.
2. Free fatty acid in original oil.
3. The buffer material.
4. The enzyme itself.

It is the quantity of the first of these that we need to calculate the lipolytic activity. From the total titration, we must subtract the others. The second and third are constant for all members of the series and are eliminated from the calculation in the subtraction of the blank. The correction for the enzyme is found by titrating a solution containing 500 milligrams of enzyme with N/10NaOH. In the test cited as an example, 500 milligrams of enzyme consumed 4.7 cc. of N/10NaOH, making a correction of 0.0094 cc. per milligram of enzyme.

It is also necessary to know what titration would result from complete hydrolysis of the oil. This is obtained by dividing the Koettendorfer saponification number, obtained in the usual way, by the ratio  $5.61/2.5 = 2.244$ . 5.61 is the number of milligrams of KOH equivalent to 1 cc. and 2.5 is the weight of oil in the test. The titration of total fatty acid thus equals  $185.5/2.244 = 82.7$  cc. The ratio of the corrected titration for the digestion mixture to 82.7 cc. gives the fraction of oil hydrolyzed by the enzyme.

The following gives an example.

TITRATION (cc. N/10 NaOH)

Milligrams Enzyme	Gross	Less Blank	Corrected for Enzyme (t)	Percent Hydrolyzed (100t/82.7)
none	7.6	0.0	0.0	0.0
10	9.0	1.4	1.4	1.7
50	11.7	4.1	3.6	4.4
100	18.5	10.9	10.0	12.1
200	30.8	23.2	21.3	25.7
500	44.0	36.4	31.7	38.3

Values for per cent hydrolyzed are now plotted against milligrams of enzyme and a smooth curve drawn. A point is marked on the

curve corresponding to a digestion of 24 per cent. This occurs at 0.19 gram of enzyme. Therefore the lipolytic activity  $= 1/g = 5.2$ . In this, as well as in the other methods described, the accuracy may be increased to any desired extent by repeating the test and using closer intervals of enzyme strength.

*Wohlgemuth Method.*<sup>83</sup> The lipolytic activity is defined as the number of cubic centimeters of tenth-normal sodium hydroxide required to neutralize the free fatty acids liberated from 10 grams of emulsified olive oil by 0.10 gram of the sample in 2 hours at 40° C.

In studying the method, Wilson and Merrill<sup>81</sup> used the following directions. Weigh off three 10-gram samples of pure olive oil into small flasks, add a drop of phenolphthalein indicator solution to each and titrate with 0.25-normal NaOH solution, with vigorous stirring, until a faint pink color persists. Place the flasks in the thermostat at 40° C. At an interval of about 5 minutes, add 0.1 gram of the sample to flasks 1 and 2, stirring vigorously, and noting time of addition. Stir every 15 minutes during the total digestion period of 2 hours. Treat another 0.1-gram sample with 5 cc. of water kept near the boiling point for 15 minutes in order to inactivate the enzyme, cool, and add it to flask 3 and digest for 2 hours.

At the end of the digestion period, add to each flask 30 cc. of a mixture of 1 part of neutral ether to 5 parts of neutral alcohol and titrate with 0.1-normal NaOH to a permanent pink endpoint. The titrations from the two tests with active enzyme are averaged. The titration from the test with inactivated enzyme is subtracted and the difference represents the value for lipolytic activity.

The method has the advantage of speed, but for greater accuracy, the Willstaetter method is to be preferred.

### Comparative Analyses.

The seven methods described above for determining enzyme activities were used by Wilson and Merrill<sup>82</sup> in the study of nine commercial samples of pancreatic enzymes offered as bating materials. Their results are given in Table XXIX.

The enzyme preparations are listed in order of increasing value by the Fuld-Gross method for casein because this method is probably used more widely than the others for the commercial evaluation of pancreatin. The Northrop values follow the same general order, but quantitatively they are different. For example, the Fuld-Gross method indicates that No. 3 is more than three times as strong as

TABLE XXIX

Pancreatin No.	Casein (F-G)	Casein (N)	Activity Upon				
			Collagen	Elastin	Keratose	Fat (Wi)	Fat (Wo)
1	3	7	4.3	40	24	17.2	46.5
2	7	14	3.3	11	4.2	3.4	1.2
3	22	14	1.8	10	4.5	4.0	18.5
4	33	52	6.1	17	11.5	4.4	10.5
5	60	114	12.1	25	23.3	5.2	53.0
6	83	133	13.3	20	27.3	1.8	31.0
7	167	308	31.3	20	45	4.3	29.5
8	143	—	30.0	0.4	86	1.4	0.5
9	333	770	38.5	50	133	0.9	0.7

No. 2, but Northrop's method shows them to be of equal strength. The Northrop method is really the more reliable, because it includes control of pH value and uses as end-point the digestion of only a fraction of the casein.

The figures for keratose run in the same order as those for casein, but lack of quantitative agreement would indicate that this is partly due to chance. The order of the collagen figures differs considerably from that of the casein figures, while the elastin figures bear no relation at all to any of the others.

In the fat columns, the Willstaetter figures are to be preferred to those obtained by the Wohlgemuth method because the Willstaetter method provides for a better control of pH value and gives results more easily reproducible. Both methods show clearly that the pancreatins used in bating do possess marked fat-splitting powers, but the variations in proteolytic activities suggest that it would be unwise to accept the figures obtained for olive oil as a quantitative measure of the activities on the skin fats.

This work has brought out clearly the following important facts:

(1) The individual enzymes making up a commercial pancreatin may be present in very variable proportions.

(2) In measuring the activity of pancreatin upon some particular material, that material itself, and not some substitute, must be used as substrate.

(3) In determining the value of a pancreatin for bating, it is necessary to measure its activity upon each important constituent of the skin to be bated.

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## Chapter II.

### Drenching and Pickling.

In the final preparation of the skin for tanning, the pH value of the solution absorbed by the skin and with which the skin is in equilibrium must be adjusted to suit the particular method of tanning to be employed. During liming, this solution has a pH value of about 12.5; during bating, a pH value of about 7.5. Before skins can be tanned properly by any of the common methods of tanning, the pH value of this solution must be lowered considerably below the value 7.5. During vegetable tanning, the pH value of the liquor is usually less than 5 and in chrome tanning less than 4. By using tan liquors containing the proper excess of acid, the adjustment of pH value may be made in the tan liquor itself. But this is often a very difficult matter where the process is not under rigid chemical control.

#### Drenching.

For certain classes of leather, it is customary to subject the bated skins, before tanning, to a process known as *drenching*. Sometimes the bating process is omitted, as unnecessary, and the skins are drenched directly after the washing following the unhairing process. The drench liquor is prepared by mixing 5 to 10 grams of bran per liter of water at 30° to 35° C. and allowing the mixture to ferment, with the formation of organic acids. The skins are put into this liquor contained in a vat equipped with a paddle wheel which keeps the liquor well stirred. In some tanneries, the fermentation is carried out in special tanks and only the clear, decanted, acid solution used on the skins. The acid dissolves any lime remaining in the skin and brings the skin into a more suitable condition for tanning. The particles of bran also exert a sort of cleansing action upon the skin, tending to absorb dirt and greases. The treatment is usually continued for several hours, but the completion of the process is determined by skilled workmen, who have learned to judge by the feel and appearance of the skin just when it is ready for the particular tanning process to be employed.

During the process, there is a considerable evolution of gas, which tends to cause the skins to float to the surface. In a drench in actual use, Wood <sup>2</sup> found that the gases had the following composition:

Carbon dioxide	25.2 per cent
Hydrogen sulfide	trace
Oxygen	2.5
Hydrogen	46.7
Nitrogen	26.0

The acids produced per liter were

Formic	0.03 gram
Acetic	0.20
Butyric	0.01
Lactic	0.79

Only an insignificant quantity of other materials were formed during drenching, trimethylamine being the chief.

It was found that the starch of the bran is converted into glucoses and dextrin by the action of an amylolytic enzyme, cerealin, discovered by Mege Mouriés.<sup>3</sup> It resembles the diastase of translocation described by Brown and Morris<sup>1</sup> in their work on the germination of grass seeds. It transforms starch into dextrin and glucose, whereas ordinary malt diastase transforms starch into dextrin and maltose. The action of cerealin is much slower than that of diastase. The sugars are then fermented by bacteria (*Bacillus furfuris*) with the formation of the organic acids listed above. The principal acid produced is lactic; the acetic acid is produced directly from the glucoses without any preliminary alcoholic fermentation by yeasts.

In the hands of experienced operators, the drenching process seldom gives much trouble, but it is not quite foolproof. If the acidity of the liquor increases rapidly and the skins are not removed in time, they become excessively swollen and may even be destroyed by hydrolysis, especially if the liquor is very warm. How much enzymes play a part in this hydrolysis is not yet known. Apparently danger from this source can be prevented by adding salt to the liquor to repress the swelling of the skin just as soon as it becomes very noticeable.

J. S. Rogers<sup>4</sup> studied the effect of lactic and acetic acids upon the plumping of hide substance and found a maximum degree of plumping at a pH value of 2.3, whichever acid was used. The acid plumping was decreased by addition of tannin.

In his review of the damage to skins that may be caused by im-

proper control of the drenching operation, Wood<sup>5</sup> points out that the discovery of the effectiveness of salt in preventing the destruction of skin in an acid liquor that would otherwise cause excessive swelling represents the origin of the modern pickling process.

Sometimes the fermentation may not proceed in the usual manner and the liquor, instead of becoming acid, turns slightly alkaline, frequently becoming bluish black, due to the presence of chromogenic bacteria. Under these conditions the skin is rapidly attacked by proteolytic organisms, but may be saved if transferred in time to a solution of acid and salt.

When the fermentation is accompanied by a very rapid evolution of gas, the skins may be damaged by the formation of gases inside of the skin which burst out through the grain surface, leaving small holes. A damage very similar in appearance may be caused by proteolytic bacteria developing on the grain surface, each colony forming a small hole. This usually results from operating the drench at too high a temperature. A high temperature, especially in the presence of an excess of acid over that normally present, may result in a considerable amount of hydrolysis of collagen and the leather will feel rather spongy and empty.

When bacteria attack the grain during drenching, the surface of the finished leather may show dull patches, as though it were etched. In one instance, Eitner<sup>2</sup> found that this was caused by *Bacillus megaterium*, which formed a slimy film over the grain surface, which was attacked by a proteolytic enzyme secreted by the bacillus.

Wood and Wilcox<sup>7</sup> showed that if the acids ordinarily found in the drench are used in pure solution in the proportions in which they occur in the drench, the action upon the skin is the same, except for being more rapid. With the appreciation of the fact that the active constituent of the drench is the acid formed, tanners began to substitute pure solutions of organic acids, such as lactic and acetic. These could be used with safety, simply by adding the acid at such rate as to keep the solution just neutral to methyl orange. Hydrochloric acid, being cheaper, is often used, although it makes the control more delicate. In this way practically all of the lime can be removed from the skins and the skins then combine with a sufficient amount of the acid so that they do not reduce the acidity of the ordinary vegetable tan liquor into which they may be put.

But even when pure solutions of acid were employed to drench skins, no fixed rule could be made for all tanneries. If the vegetable tan liquors contained a considerable amount of salt and other soluble

nontannins, the drench could be operated at a lower pH value with safety. Where fresh liquors of tanning materials containing a relatively small proportion of nontannin were used, there was danger of the skins being damaged by the rhythmic swelling described in Chapter 5, whenever the pH value of the drench fell below some fixed value, which depended upon the composition of the tan liquor employed. This trouble can be avoided by the addition of salt to the tan liquor, but the remedy may be almost as undesirable as the disease, since many tan liquors are precipitated by the addition of salt. In general, the purer the first tan liquor into which the skins are put after drenching, the more delicate must the control of the drenching operation be.

It sometimes happens that the tan liquors employed contain easily fermentable sugars, which are continually being converted into organic acids. In such cases, the use of a drench prior to tanning may be undesirable. The tan liquor itself actually becomes a drench and the lime salts formed serve to prevent rhythmic swelling. Where the skins have been drenched prior to putting into the tan liquor, the acid present may prove excessive and the skins will be spoiled.

One tanner may employ a tan liquor of low acidity preceded by a drench, another may use tan liquors of greater acidity and do away with the drenching operation, and yet both may produce the same kind of finished leather. But one would not dare to adopt only a part of the other's methods, for that might prove disastrous; he must adopt all or none. This will serve to explain why it is not possible to outline quantitatively a rigid system of bating, drenching, deliming, or any other process, so that it may be used in any tannery. All fundamental operations in any one tannery are interdependent and a change, even one for the better, in one operation might necessitate a corresponding change in nearly every other operation.

### **Pickling.**

The pickling operation differs from drenching chiefly in the fact that salt is used in conjunction with the acid. Formerly it was the customary practice to soak the limed or bated skins in a vat containing dilute sulfuric acid until they became somewhat swollen and then to transfer them to a saturated solution of sodium chloride, which repressed the swelling. Now it is more common to use the acid and salt in solution together, the preliminary swelling having been found unnecessary and sometimes undesirable. A satisfactory pickle liquor

for most purposes consists merely of a molar solution of sodium chloride to which sulfuric acid is added in the desired amounts.

Pickle liquors are used for a number of different purposes, the chief of which are the preparation of skin for chrome tanning and the preservation of unhaired skins so that they may be kept for an indefinite period before tanning.

In preparing skins for chrome tanning, the concentration of acid most desirable to use depends upon the degree of basicity of the chrome liquor employed. The more concentrated the acid in the pickle liquor, the more quickly does the system tend to reach a condition approximating equilibrium. Furthermore, the more concentrated the acid solution absorbed by the skin, the more quickly will the chromium salts penetrate into the interior of the skin during the tannage. On the other hand, if the concentration of acid is too great, the rate of fixation of chromium by the skin will be reduced to an undesirable degree, unless the excess of acid is neutralized by the addition of sodium bicarbonate, borax, or other agent, during the tannage.

Pickling has the advantage over drenching that it is extremely easy to control chemically. If the concentration of salt is not allowed to fall below half-molar, the pickle liquor can be controlled by simple titrations, using methyl orange as indicator. Regardless of the variable amounts of lime which the skins may contain before pickling, they can all be brought into a uniform condition simply by so regulating the concentration of acid that all skins finally reach equilibrium with solutions of the same concentration. When used in this way, the pickling process becomes a stabilizer of inestimable value in chrome tanning.

When the equilibrium concentration of acid is maintained at 0.05 normal or greater, the pickling of light skins requires only a few hours, but for weaker solutions and for heavy hides, the stock must remain in the liquor over night. In acid solutions greater than 0.01 normal, there is practically no danger of the skins being attacked by bacteria. The salt present is sufficient to prevent undue swelling at any pH value so that the process may be considered entirely safe, if only ordinary care is used.

For preserving skins, after bating, it is sufficient to bring them into equilibrium with a solution containing 1 mole of sodium chloride and 0.01 mole of sulfuric acid per liter. The liquors may be used for several consecutive lots of skin as the calcium sulfate formed is soluble in acid solution. The skins are usually pickled in vats equipped with paddle wheels, which keep the skins and liquor in motion, greatly

hastening the attainment of equilibrium. After equilibrium has been established, the skins are withdrawn from the liquor and thrown over wooden horses to drain. They may then be kept in a damp condition for many months.

It is often desired to tan such skins later in vegetable tan liquors of such composition that they would be precipitated by the salt and acid present in the skins. In such cases, the skins are first depickled by soaking in paddle vats containing a solution of half-molar sodium chloride to which borax is added at such rate as to keep the solution neutral to methyl orange. When equilibrium has been established, the skins are transferred to a wash wheel and the salt washed out by means of running water. They are then ready for tanning. Depickling is unnecessary in the case of chrome tanning.

In the control of pickle liquors, it must not be assumed that the decrease in concentration of acid is caused only by its neutralization by lime. Two other factors contribute to the decrease. The bated skins usually contain about 80 per cent by weight of water, only 20 per cent representing collagen. Part of the decrease is caused by the dilution by this water. The author has found that 1 gram of collagen combines with approximately 0.00133 gram equivalent of acid. By making allowance for the decrease in concentration of acid caused by dilution and by combination with the collagen, the amount consumed in neutralizing lime can be roughly approximated.

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## Chapter 12.

### Vegetable Tanning Materials.

It has been known since prehistoric times that raw skin is colored and rendered imputrescible by contact with aqueous solutions of materials obtained from many forms of plant life. The active principle, which is widely\* distributed throughout the vegetable kingdom, is a class of complex organic compounds known as *tannin*. By vegetable tanning is meant the combination of tannin with the protein matter of skin to form leather.

Among the materials which have assumed commercial importance as a source of tannin for leather manufacture are barks, woods, leaves, twigs, fruits, pods, and roots. Tanning extracts obtained from different sources show very different properties, which are due in a large measure to the foreign matter extracted with the tannin. Certain plant cells are very rich in tannin. In the living cell, the tannin occurs dissolved in the cell sap and associated with other substances, including carbohydrates and salts. Lloyd<sup>o</sup> has indicated evidence that the carbohydrates are able to link up with the tannin in some way so as to prevent its attack upon the living protoplasm. The tannin appears to be useful in some way in the metabolism of the plant.

#### Sources of Tannin Materials.

Wilson and Thomas<sup>11</sup> have compiled a list of natural sources of tannin, arranged alphabetically according to the botanical name. This list is reproduced in Table XXX. The names and tannin contents were taken from the literature at large for what they may be worth; in some cases the information given may be considerably in error. The place grown may indicate either the place where the sample analyzed was grown or the place where the material grows in abundance. In the majority of cases, at least, the tannin contents are supposedly those of the air-dried material.

In using the tannin figures, it should be recognized that they are not true tannin contents, but merely figures obtained by methods open

**TABLE XXX**  
**SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS**

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Abies alba</i> .....	White spruce	Northern America	Bark 7-13
<i>Abies canadensis</i> .....	Hemlock fir	Northern America	Bark 8-15
<i>Abies dumosa</i> .....	Hemlock spruce	Northern America	Bark 10
<i>Abies ercelsa</i> .....	Norway spruce	Northern Europe	Bark 7-13
<i>Abies grandis</i> .....	Lowland fir	California	Bark 9
<i>Abies pectinata</i> .....	Silver fir	Europe	Bark 6-15
<i>Acacia acuminata</i> .....	Raspberry jam wood	Australia	Bark 4-15
<i>Acacia angica</i> .....	Angica	Brazil	Bark 20-25
<i>Acacia anema</i> .....	Mulga	New South Wales	Bark 5-9
<i>Acacia arabica</i> .....	Babul	India	Bark 12-20
<i>Acacia binervata</i> .....	Black wattle	Australia	Pods 20-42
<i>Acacia brachybotrya</i> .....	Blue bush	New South Wales	Bark 27-30
<i>Acacia catechu</i> .....	Cutch	India	Bark 21
<i>Acacia cavenia</i> .....	Espinillo	South America	Wood ext. 60
<i>Acacia cebil</i> .....	Red cebil	Argentina	Pods 18-21
<i>Acacia cunninghamii</i> .....	Pea wattle	Queensland	Bark 6
<i>Acacia curup</i> .....	Curup	South America	Bark 10-15
<i>Acacia dealbata</i> .....	Silver wattle	Australia, Africa and Asia	Leaves 6-7
<i>Acacia decora</i> .....	Blue bush	New South Wales	Bark 9-18
<i>Acacia decurrens</i> .....	Green wattle	Australia	Bark 18
<i>Acacia elata</i> .....	Mountain hickory	New South Wales	Bark 14-32
<i>Acacia falcata</i> .....	Stunted wattle	Queensland	Bark 14-23
<i>Acacia flavescens</i> .....	Red wattle	Queensland	Bark 18-51
<i>Acacia granulosa</i> .....	Yarran	New Caledonia	Bark 20-31
<i>Acacia homalophyla</i> .....	Doornbosch	New South Wales	Bark 13-37
<i>Acacia horrida</i> .....	Koa tree	Cape Good Hope	Bark 19-22
<i>Acacia koa</i> .....		Hawaii	Bark 12
<i>Acacia leptocarpa</i> .....		Queensland	Bark 9
			Bark 8-18
			Bark 18
			Bark 10



SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Acacia longifolia</i> .....	Wild willow	Cyprus and Australia	Bark 7-19
<i>Acacia melanoxylon</i> .....	Blackwood	New South Wales	Bark 11-13 Leaves 3
<i>Acacia microbotrya</i> .....	Manna wattle	Australia	Bark 18-27 Leaves and twigs 20
<i>Acacia mollissima</i> .....	Green wattle	Australia	Bark 12-47
<i>Acacia nerifolia</i> .....	Black wattle	Australia	Bark 14
<i>Acacia oswaldi</i> .....	Miljie	Australia	Bark 10
<i>Acacia penninervis</i> .....	Hickory wattle	Europe and Australia	Bark 14-38
<i>Acacia podalyraefolia</i> .....	Silver-leaved wattle	Queensland	Bark 8-21
<i>Acacia polytachya</i> .....	Golden wattle	Queensland	Bark 18
<i>Acacia pycnantha</i> .....	Willow wattle	Australia	Bark 26-50
<i>Acacia salicina</i> .....	Weeping willow	New South Wales	Bark 6-20
<i>Acacia saligna</i> .....	Thorny wattle	New South Wales	Bark 28
<i>Acacia sentis</i> .....	Talh	Sudan	Bark 6-18
<i>Acacia seyal</i> .....	Gallol	Somaliland	Bark 18
<i>Acacia sp.</i> .....	Guaiac	New Caledonia	Bark 24
<i>Acacia spiralis</i> .....	Himalayan maple	India	Bark 17
<i>Acer campestre</i> .....	Field maple	Europe	Bark 3
<i>Achornea triplinervia</i> .....	Tapia gwazu-ih	Paraguay	Bark 4
<i>Allophylus edulis</i> .....	Koku	Paraguay	Bark 12
<i>Alnus firma</i> .....	Minibari	Japan	Bark 10
<i>Alnus glutinosa</i> .....	Alder	Europe	Fruits 25
<i>Alnus incana</i> .....	Grey alder	Europe	Bark 16-20
<i>Alnus maritima</i> .....	Hannoki	Japan	Bark 10
<i>Alnus oregona</i> .....	Red alder	Pacific states	Fruits 25
<i>Anacardium occidentale</i> .....	Kashew nut	India	Bark 9
<i>Angophora intermedia</i> .....	Rough-barked apple	New South Wales	Bark 4-5 Kino 65
<i>Angophora lanceolata</i> .....	Smooth-barked apple	Australia	Bark 6-11
<i>Anogeissus acuminata</i> .....	Yon	India	Kino 61-68 Bark 10

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Anogeissus latifolia</i> .....	Dhawa	India	Bark 16 Leaves 10-18 Shoots 20-30 Red tips 54
<i>Anogeissus pendula</i> .....	Yvithra-pere	India	Bark 9
<i>Apuleia praecox</i> .....	Bearberry	Paraguay	Bark 11
<i>Arctostaphylos uva-ursi</i> .....	Betelnut palm	Russia	Leaves and twigs 14
<i>Arca catechu</i> .....	Palo rosa	India	Fruits 10-15
<i>Aspidosperma polyneuron</i> .....	White quebracho	Paraguay	Bark 3
<i>Aspidosperma quebracho-blanco</i> .....	White mangrove	Argentina	Leaves 27-28 Bark 4
<i>Avicennia officinalis</i> .....	Coast honeysuckle	Queensland	Wood 3
<i>Banksia integrifolia</i> .....	Heath honeysuckle	Queensland	Bark 4-24
<i>Banksia serrata</i> .....	Muhurin bark	Australia	Bark 6-11
<i>Bauhinia vahlii</i> .....	White birch	India	Bark 11-23
<i>Betula alba</i> .....	Salai bark	India	Bark 9
<i>Betula lenta</i> .....	Black mangrove	Northern Europe	Bark 2-18
<i>Boswellia serrata</i> .....	Hagalay	Northern America	Bark 3-18
<i>Bruguiera gymnorhiza</i> .....	Red mangrove	India	Bark 13
<i>Bruguiera parviflora</i> .....	Mangrove	East Africa and Australia	Bark 22-52
<i>Bruguiera rheedi</i> .....	Pilkasurembiu	Philippines	Bark 7-13
<i>Bruguiera rumphii</i> .....	Mureci	Queensland	Bark 15-22
<i>Bumelia obtusifolia</i> .....	Tamwood	New Caledonia	Bark 27-42
<i>Byrsonima cydoniaefolia</i> .....	Cancharana	Paraguay	Root bark 6 Root wood 9
<i>Byrsonima spicata</i> .....	Algarobilla	Bolivia	Bark 8
<i>Cabralea</i> sp. ....	Cascalote	South America	Bark 20
<i>Caesalpinia brevifolia</i> .....	Divi-divi	Paraguay	Bark 44
<i>Caesalpinia cocolaco</i> .....	Tari	Chile	Bark 5
<i>Caesalpinia coriaria</i> .....		Mexico	Pods 43-67
<i>Caesalpinia digyna</i> .....		Central America	Pods 40-55
		India and Burma	Pod cases 40-60

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Caesalpinia melanocarpa</i> .....	Guyacan	Argentina	Pods 15-23
<i>Caesalpinia tinctoria</i> .....	Celavania	Central America	Wood 8
<i>Callitris calcarata</i> .....	Black cypress pine	Australia	Pods 30-32
<i>Callitris glauca</i> .....	Cypress pine	Australia	Bark 12-34
<i>Camellia thea</i> .....	Tea	Asia and Africa	Bark 11-24
<i>Carapa moluccensis</i> .....	Orange mangrove	Queensland	Leaves 5-10
<i>Carissa spinarum</i> .....	Tarwar	India	Bark 23-34
<i>Cassia auriculata</i> .....	Amaltas	India	Leaves 8-12
<i>Cassia fistula</i> .....	Spanish chestnut	South India	Bark 16-22
<i>Castanea cerva</i> .....		{ Southern Europe	Bark 11-15
		{ Southern U. S.	Pod husk 17
			Bark 6-8
			Wood 7-11
			Stump and trunk bark 13
			Stump heartwood,
			center 9
			intermediate 13
			edge 16
			Root wood 17
			Root bark 31
			Trunk heartwood,
			center 9
			edge 15
			Bark 6
			Wood 7
			Bark 8
			Bark 12
			Bark 10
			Bark 11-18
			Bark 12-15
			Leaves 17
			Bark 24-42
			Bark 13
<i>Castanea pubinervis</i> .....	Japanese chestnut	Japan	
<i>Castanopsis chrysophylla</i> .....	Western chinquapin	Pacific states	
<i>Castanopsis sinensis</i> .....	Gie-gay	Indo-China	
<i>Casuarina</i> .....	Ironwood	New Caledonia	
<i>Casuarina equisetifolia</i> .....	Casagha pine	Southern Asia	
<i>Casuarina glauca</i> .....	Bull oak	New South Wales	
<i>Ceanothus velutina</i> .....	Snow bush	Western U. S.	
<i>Ceriops candolleana</i> .....	Bahau or mangrove	Australia, India and Africa	
<i>Ceriops roxburghiana</i> .....	Goran	India	

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Ceraps tagal</i> .....	Tangel	Philippines	Bark 24-37
<i>Cleistanthus colinus</i> .....	Kodarsi	.....	Bark 33
<i>Cocos romanzoffiana</i> .....	Pindo	.....	Bark 7
<i>Copaifera lansdorffii</i> .....	Kupah	Paraguay	Bark 17
<i>Coriaria myrsifolia</i> .....	French sumac	France	Leaves 15
<i>Coriaria nepalensis</i> .....	.....	India	Leaves 20
<i>Coriaria ruscifolia</i> .....	Tutu	New Zealand	Bark 16-17
<i>Corylus avellana</i> .....	Hazel	Europe	Bark 5
<i>Couleria tinctoria</i> .....	Tara	Algeria and Peru	Pods 43-51
<i>Crossostylis multiflora</i> .....	Bush mangrove	New Caledonia	Wood 21
<i>Cryptomeria japonica</i> .....	Japanese cedar	Japan	Bark 3
<i>Cupania sp.</i> .....	Cedrillo	Paraguay	Bark 6
<i>Cupania uraguensis</i> .....	Kambuata	Paraguay	Bark 16
<i>Cupania vernalis</i> .....	Yaguataih	Paraguay	Bark 18
<i>Dalbergia sp.</i> .....	Yhsaph-ih	Paraguay	Bark 15
<i>Dioscorea atropurpurea</i> .....	Cu-nao	Indo-China	Bark 6
<i>Elaeocarpus grandis</i> .....	Blue fig bark	New South Wales	Tubers 20
<i>Elephantorrhiza burchelli</i> .....	Elephant roots	Africa	Bark 10
<i>Enterolobium timbouva</i> .....	Timbo	Paraguay	Root 6-22
<i>Eremophila longifolia</i> .....	Emu bush	New South Wales	Bark 22
<i>Eucalyptus accedens</i> .....	Spotted gum	Australia	Bark 5
<i>Eucalyptus alba</i> .....	Mountain gum	Australia	Leaves 10
<i>Eucalyptus amygdalina</i> .....	Ribbon gum	New South Wales	Bark 18
<i>Eucalyptus campaspe</i> .....	Silver-topped gimlet	Australia	Bark 30-32
<i>Eucalyptus corymbosa</i> .....	Bloodwood	New South Wales	Kino 58-65
<i>Eucalyptus corynocalyz</i> .....	Sugar-gum	Australia	Bark 27
<i>Eucalyptus diversicolor</i> .....	Karri	Australia	Kino 63-69
<i>Eucalyptus erythronema</i> .....	White mallet	Australia	Leaves 18
<i>Eucalyptus falcata</i> .....	Silver mallet	Australia	Bark 21-28
			Bark 16-20
			Bark 30
			Bark 5-32

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Eucalyptus gardneri</i> .....	Blue-leaved mallet	Australia	Bark 23-31
<i>Eucalyptus globulus</i> .....	Eucalyptus	Australia	Sap 28
<i>Eucalyptus gunnii</i> .....	Red gum	New South Wales	Leaves 17
<i>Eucalyptus longifolia</i> .....	Woolly-butt	Australia	Bark 11
<i>Eucalyptus lorophleba</i> .....	York gum	Australia	Bark 2-16
			Bark 5-10
<i>Eucalyptus maculata</i> .....	Spotted gum	New South Wales	Kino 37-45
			Bark 3-10
<i>Eucalyptus obliqua</i> .....	Stringy bark	New South Wales	Leaves 5
<i>Eucalyptus occidentalis</i> .....	Black mallet	Australia	Bark 2-17
<i>Eucalyptus occidentalis astrigens</i> .....	Red mallet	Australia	Bark 20-26
<i>Eucalyptus odorata</i> .....	White box	New South Wales	Bark 34-57
<i>Eucalyptus pallidifolia</i> .....	Micum	Australia	Leaves 7
<i>Eucalyptus paniculata</i> .....	Grey ironbark	Australia	Bark 28
			Bark 8-30
<i>Eucalyptus piperita</i> .....	Messmate	Australia	Kino 72-83
			Kino 32-62
<i>Eucalyptus platypus</i> .....	Round leaf moort	New South Wales	Leaves 13
<i>Eucalyptus redunda</i> .....	Wandoo	Australia	Bark 25-29
<i>Eucalyptus resinifera</i> .....	Stringybark	Australia	Bark 16-20
<i>Eucalyptus redunda oxymitra</i> .....	Blue leaf mallet	Australia	Bark 1-6
<i>Eucalyptus robusta</i> .....	Mahogany	Florida	Kino 74
			Bark 22-30*
<i>Eucalyptus rostrata</i> .....	Red gum	Australia	Leaves 12-17
			Bark 16
<i>Eucalyptus salomonophloia</i> .....	Salmon gum	Australia	Kino 30-83
<i>Eucalyptus salubris</i> .....	Gimlet	Australia	Wood 2-14
			Bark 8-20
<i>Eucalyptus siderophloia</i> .....	Red iron bark	Australia	Bark 16-19
			Kino 35-73
			Bark 7-13
			Leaves 6

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Eucalyptus sideroxylon</i> .....	Ironbark	New South Wales	Bark 16-33
<i>Eucalyptus sieberiana</i> .....	Mountain ash	New South Wales	Kino 44
<i>Eucalyptus smithii</i> .....	Gully ash	New South Wales	Bark 5-37
<i>Eucalyptus spathulata</i> .....	Swamp gimlet	Australia	Bark 21-28
<i>Eucalyptus stellulata</i> .....	Black gum	New South Wales	Bark 26
			Bark 13
			Leaves 17
<i>Eucalyptus stuartiana</i> .....	Apple	New South Wales	Bark 3-5
<i>Eucalyptus torquata</i> .....	Flowering gum	Australia	Leaves 10
			Bark 17
<i>Eucalyptus viminalis</i> .....	Manna gum	New South Wales	Bark 4-8
			Kino 69
			Leaves 4
<i>Eugenia brazilensis</i> .....	Yhva-poroitih	Paraguay	Bark 43
			Leaves 17
<i>Eugenia jambolana</i> .....	Java plum	India	Wood 12
<i>Eugenia jambos</i> .....		Brazil	Bark 19
<i>Eugenia maire</i> .....		New Zealand	Bark 12
<i>Eugenia michellii</i> .....	Nangapirih gwazu	Paraguay	Bark 16-17
<i>Eugenia pungens</i> .....	Yhva viyu	Paraguay	Bark 29
<i>Eugenia smithii</i> .....		Australia	Bark 11
<i>Eugenia</i> sp. ....	Yhvahay puihta gwazu	Paraguay	Bark 17
<i>Erocarpus cupressiformis</i> .....	Native cherry	Paraguay	Bark 16-29
<i>Ficus</i> sp. ....	Kili bark	Australia	Bark 15-23
<i>Fusanus acuminatus</i> .....	Quandony	Sudan	Bark 19
<i>Garicinia mangostana</i> .....	Mangoustan	Australia	Bark 19
<i>Grevillia striata</i> .....	Beefwood	Cochin-China	Fruit shells 14
<i>Guarea</i> sp. ....	Guare	Australia	Bark 18
<i>Hakea glabella</i> .....	Prickly pear	Paraguay	Bark 10
<i>Hakea leucomptera</i> .....	Needle bark	Australia	Bark 18-20
<i>Heritiera fomes</i> .....	Sundri bark	New South Wales	Bark 11
		India	Bark 7

Source and Tannin Content of Vegetable Tanning Materials.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Hopea odorata</i> .....	.....	India	Bark 14-15 Leaves 11
<i>Hopea parviflora</i> .....	Ironwood	India	Wood 10
<i>Hydnora longicollis</i> .....	Gamb	Africa	Bark 17-22
<i>Inga affinis</i> .....	Inga gwazu	Paraguay	Roots 32
<i>Inga feuillei</i> .....	Paypay	Peru	Bark 26
<i>Juniperus recurva</i> .....	Weeping blue	Japan	Pods 12-15
<i>Krameria triandra</i> .....	Rhatany	Peru	Bark 8
<i>Larix dahurica</i> .....	Larch	Japan	Root bark 20
<i>Larix europaea</i> .....	Larch	Europe	Bark 9
<i>Larix occidentalis</i> .....	Western larch	N. W. United States	Bark 9-10 Bark 11 Wood 7
<i>Laurus lingue</i> .....	.....	Chile	Bark 17-19
<i>Leuceadendron argenteum</i> .....	Silver tree	Cape Good Hope	Bark 9-16
<i>Leucospermum conocarpum</i> .....	Knotted tree	Cape Good Hope	Bark 10-22
<i>Ludwigia caparossa</i> .....	Caparossa	Brazil	Bark 20-25
<i>Lysiloma candida</i> .....	Palo blanco	Lower California	Bark 26
<i>Maclura pomifera</i> .....	Osage orange	Texas	Wood 11
<i>Malpighia laginca</i> .....	Nance	Mexico	Bark 26
<i>Malpighia punicifolia</i> .....	Mangrutta	Nicaragua	Bark 20-30
<i>Mimosa farinosa</i> .....	Mimosa	Argentina	Bark 4
<i>Mimosa pudica</i> .....	Mimosa	India	Roots 10
<i>Mimosa</i> sp. ....	Yukeri gwazu	Paraguay	Bark 11
<i>Myrica asplenifolia</i> .....	Sweet fern	Michigan	Leaves 4-5 Roots 4-6
<i>Myrica nagi</i> .....	Box myrtle	India	Roots 4-6
<i>Nauclea gambir</i> .....	Gambier	East Indies	Bark 13-27
<i>Ocotea bullata</i> .....	.....	South Africa	Leaves and twigs 5-6
<i>Ocotea</i> sp. ....	Yhva-lha	Paraguay	Bark 6
<i>Ostrya abyssinica</i> .....	.....	Transvaal	Bark 11
<i>Ostrya arborea</i> .....	.....	Northern India	Leaves and twigs 13-25
<i>Ostrya compressa</i> .....	Cape sumac	Cape Good Hope	Leaves 20 Leaves 17-23

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Oralis gigantea</i> .....	Guara	Chile	Bark 25
<i>Paulinia sorbilis</i> .....	Yhvihra puihta	Brazil	Fruit 43-55
<i>Peltophorum dubium</i> .....		Paraguay	Bark 31
<i>Pentacme suavis</i> .....		India	Leaves 12-24 Bark 7-13 Wood 4
<i>Phyllanthus emblica</i> .....	Amla	India	Stoned fruit 26-35 Leaves 23-28
<i>Phyllocladus asplenifolia</i> .....	Celery-topped pine	Tasmania	Bark 15-24
<i>Phyllocladus rhomboidalis</i> .....	Celery-topped pine	Tasmania	Bark 23
<i>Phyllocladus trichomanoides</i> .....	Red yezomatsu	New Zealand	Bark 15-21
<i>Picea glehni</i> .....	Sitka spruce	Japan	Bark 28-30
<i>Picea sitchensis</i> .....	Pine	Pacific states	Bark 19
<i>Pinus cembra</i> .....	Red pine	Alpine Europe	Bark 12-18
<i>Pinus densiflora</i> .....	Aleppo pine	Japan	Bark 3-5
<i>Pinus halepensis</i> .....	Pine	Mediterranean coasts	Bark 6
<i>Pinus Khasya</i> .....	Long-leaved pine	Burma	Bark 10-20
<i>Pinus longifolia</i> .....	Swamp pine	India	Bark 7-10
<i>Pinus muricata</i> .....	Monterey pine	California	Bark 11-14
<i>Pinus radiata</i> .....	Scotch fir	California	Bark 13
<i>Pinus sylvestris</i> .....	Black pine	Northern Europe	Bark 4-5
<i>Piptadenia bergerii</i> .....		Japan	Bark 6
<i>Piptadenia cebil</i> .....		Argentina	Bark 15
<i>Piptadenia rigida</i> .....	Kurupah-ra puihta	Paraguay	Bark 28
<i>Pistacia lentiscus</i> .....	Pistacio	Mediterranean	Leaves 12-19
<i>Pistacia orientalis</i> .....	Camanchile	India	Galls 30-40
<i>Pithecolobium dulce</i> .....		Mexico	Bark 15-25
<i>Polygonum amphibium</i> .....		Missouri	Roots 22
<i>Polygonum bistorta</i> .....	Snakeweed	England	Branches 17
<i>Populus tremula</i> .....	Poplar	Europe	Roots 16-21
<i>Prosopis oblonga</i> .....	Abu-surug	Sudan	Bark 3 Bark 14



SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Protea grandiflora</i> .....	.....	Cape Good Hope	Bark 15-16
<i>Protea mellifera</i> .....	Sugarbush	Cape Good Hope	Bark 18-25
<i>Pseudotsuga taxifolia</i> .....	Douglas fir	Pacific states	Bark 7
<i>Punica granatum</i> .....	Pomegranate	India	Fruit rind 27-30
<i>Quebrachia lorrentzii</i> .....	Quebracho	South America	Kernel 32
<i>Quercus aegilops</i> .....	Valonia	.....	Bark 18-22
<i>Quercus agrifolia</i> .....	Live oak	Mediterranean	Wood 20-30
<i>Quercus alba</i> .....	White oak	California	Bark 6-8
<i>Quercus californica</i> .....	Black oak	Northern America	Acorns 17-40
<i>Quercus cerris</i> .....	Turkey oak	California	Bark 19
<i>Quercus chrysolepis</i> .....	Maul oak	Southern Europe	Bark 7
<i>Quercus coccifera</i> .....	Kermes oak	Pacific states	Bark 10
<i>Quercus coccinea</i> .....	Scarlet oak	Mediterranean	Galls 35
<i>Quercus densiflora</i> .....	Tanbark oak	United States	Bark 7-12
<i>Quercus dentata</i> .....	Japanese oak	California	Bark 10-18
<i>Quercus feneestrata</i> .....	.....	Japan	Bark 8
<i>Quercus garrigano</i> .....	Pacific post oak	Northern India	Bark 10-29
<i>Quercus grosseserrata</i> .....	Water oak	Pacific states	Bark 11
<i>Quercus ilex</i> .....	Evergreen oak	Japan	Wood 7
<i>Quercus incana</i> .....	.....	.....	Bark 10-16
<i>Quercus infectoria</i> .....	Aleppo	India	Bark 6-7
<i>Quercus lamellosa</i> .....	Hill oak	Turkey	Bark 9
<i>Quercus lineata</i> .....	.....	Southern Europe	Wood 2
<i>Quercus lobata</i> .....	White oak	India	Bark 5-11
<i>Quercus mirbeckii</i> .....	.....	Northern India	Bark 22
<i>Quercus pachyphylla</i> .....	Sungra katus	Algeria	Galls 24-60
		Northern India	Bark 8-10
		California	Bark 11
		Algeria	Bark 12
		Northern India	Bark 8
			Acorn cups 13-15
			Bark 12-13
			Leaves 10

Source and Tannin Content of Vegetable Tanning Materials.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Quercus prinus</i> .....	Chestnut oak	United States	Bark 9-12
<i>Quercus pseudococcinea</i> .....	Gie-quang	Indo-China	Bark 16
<i>Quercus robur</i> .....	Common oak	Europe and U. S.	Bark 9-12
			Wood 2-4
<i>Quercus rubra</i> .....	Red oak	Northern America	Twig galls 35
<i>Quercus</i> sp. ....	Gie-bob	Indo-China	Bark 4-6
<i>Quercus suber</i> .....	Cork oak	Europe	Bark 11
<i>Quercus tozae</i> .....	.....	.....	Bark 12-19
<i>Quercus velutina</i> .....	Black oak	Southern France	Bark 14
<i>Quercus wislizeni</i> .....	Highland oak	United States	Bark 6-12
<i>Rheedia brazilensis</i> .....	Pakuri	California	Bark 7-8
<i>Rhizophora conjugata</i> .....	Mangrove	Paraguay	Bark 22
		Philippines	Bark 26-32
<i>Rhizophora mangle</i> .....	Mangrove	Tropical coasts	Bark 15-42
			Leaves 22
<i>Rhizophora mucronata</i> .....	Mangrove	Australia, Asia and Africa	Bark 21-48
<i>Rhus copallina</i> .....	Sumac	United States	Leaves 17-38
<i>Rhus coriaria</i> .....	Sicilian sumac	Sicily	Leaves 25-32
<i>Rhus cotinus</i> .....	Venetian sumac	Italy	Leaves 17
<i>Rhus cotinoides</i> .....	Sumac	United States	Leaves 21
<i>Rhus glabra</i> .....	White sumac	United States	Leaves 15-25
<i>Rhus metopium</i> .....	Sumac	United States	Leaves 8
<i>Rhus myrsorensis</i> .....	.....	Southern India	Bark 20
<i>Rhus pentaphylla</i> .....	Tizra sumac	Morocco	Roots 29
<i>Rhus rhodanthema</i> .....	Deep yellow wood	New South Wales	Wood 23
<i>Rhus semialata</i> .....	Sumac	America and Asia	Bark 23
			Leaves 5
<i>Rhus succedanea</i> .....	Sumac	India	Chinese galls 70
<i>Rhus thunbergii</i> .....	.....	Cape Good Hope	Leaves 20
<i>Rhus typhina</i> .....	Virginian sumac	Virginia	Bark 28
		Europe	Leaves 10-18
<i>Robinia pseudacacia</i> .....	Black locust		Bark 2-7
			Wood 3-4

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Rollinia</i> sp. ....	Aratiku gwazu	Paraguay	Bark 4
<i>Rumex hymenosepalum</i> ....	Canagire	Mexico	Roots 25-30
<i>Rumex maritima</i> ....	Docks	Europe	Roots 22
<i>Sabal palmetto</i> ....	Cabbage palmetto	Florida	Roots 10-18
<i>Sabal serrulata</i> ....	Saw palmetto	Florida	Leaves 13
<i>Salix alba</i> ....	White willow	.....	Bark 9
<i>Salix arenaria</i> ....	Willow	Russia	Bark 13
<i>Salix caprea</i> ....	Willow	Japan	Bark 8-12
<i>Salix fragilis</i> ....	Willow	.....	Bark 9-12
<i>Salix lasandra</i> ....	Yellow willow	California	Bark 2
<i>Salix purpurea</i> ....	Willow	Japan	Bark 8
<i>Salix viminalis</i> ....	Willow	Russia	Bark 7-10
<i>Schinus molle</i> ....	Molle	Argentina	Leaves 19
<i>Sequoia sempervirens</i> ....	Redwood	Pacific states	Heartwood 4-12 Sapwood 1-2
<i>Shorea obtusa</i> ....	.....	India	Bark 1-3
<i>Shorea robusta</i> ....	Sal bark	India	Bark 9
<i>Sonneratia pagatpat</i> ....	Pagatpat	Philippines	Wood 6-7
<i>Spermolepis gummifera</i> ....	Oak gum	New Caledonia	Bark 6-15
<i>Statice cortaria</i> ....	Marsh rosemary	.....	Bark 11-12
<i>Stryphnodendron barbatimao</i> ....	Barbatimao	Brazil	Bark 17
<i>Tamarix africana</i> ....	Tamarisk	Mediterranean	Kino 43-80
<i>Tamarix articulata</i> ....	Tamarisk	Morocco	Roots 20-22
<i>Tamarix dioica</i> ....	Jhao	India	Bark 18-27
<i>Taraxacum cuspidata</i> ....	Yew	Japan	Galls 26-56
<i>Terminalia arjuna</i> ....	Kahua	India	Twigs 9
<i>Terminalia belerica</i> ....	Bedda	India	Leaves 9
<i>Terminalia catappa</i> ....	Badamier	India	Galls 43-56

SOURCE AND TANNIN CONTENT OF VEGETABLE TANNING MATERIALS.—(Continued)

Botanical Name	Common Name	Place Grown	Percent Tannin
<i>Terminalia chebula</i> .....	Myrobalan	India	Nuts 30-40
<i>Terminalia glabra</i> .....	Kumbuk	Ceylon	Bark 27-32-
<i>Terminalia mauritiana</i> .....	Jamrosa	India	Bark 30
<i>Terminalia oliveri</i> .....	Thann	Malay	Bark 31
<i>Tormentilla erecta</i> .....		Europe	Leaves 14
<i>Trichilia catigua</i> .....	Kaatigua puihta	Paraguay	Roots 20-46
<i>Trichilia hieronimi</i> .....	Kaatigua moroti	Paraguay	Bark 21
<i>Tsuga canadensis</i> .....	Hemlock	Northern America	Bark 23
<i>Tsuga heterophylla</i> .....	Western hemlock	Pacific states	Bark 7-12
<i>Umbellularia californica</i> .....	California laurel	California	Bark 9-16
<i>Vateria indica</i> .....		India	Bark 16
<i>Weinmannia glabra</i> .....	Curtidor	Venezuela	Fruit 25
<i>Woodfordia floribunda</i> .....	Itcha	India	Bark 10-13
<i>Ximenio americana</i> .....	Alimu	Sudan	Bark 27
<i>Xylia dolabriformis</i> .....	Jamba	Burma and India	Leaves 15
<i>Xylocarpus granatum</i> .....	Piagao	Africa and Asia	Bark 17
<i>Xylocarpus obovatus</i> .....	Tabique	Philippines	Bark 9-19
<i>Zizyphus nummularia</i> .....	Ber	India	Wood 4
<i>Zizyphus xylopyra</i> .....	Gothar	India	Bark 21-48
			Bark 22-25
			Bark 10
			Fruit flesh 23

to very serious question. A number of slightly different methods were used, but all conformed roughly to the following general scheme. A solution of the tanning material of concentration confined to certain limits was treated with lightly chrome-tanned hide powder for a time more than sufficient to remove all tannin from solution as indicated by the freedom of the filtered solution from materials that precipitate gelatin from solution. The decrease in concentration of all matters in solution was then taken as the measure of the tannin content. Obviously, all substances of a slightly acid nature would be removed to some extent by the hide powder and hence all figures must be high where these are present. The probable magnitude of the errors involved for some of the commoner tanning materials will be indicated in the next chapter in connection with methods for determining tannin.

It may be assumed that nearly every form of plant life contains some tannin. The list given is not complete, but it contains most of the materials cited in the literature which contain enough tannin to make them interesting as possible sources for commercial tannin. It was considered impractical to try to list the enormous number of journal articles consulted in making the compilation.

### American Survey.

The United States Department of Commerce has made an important survey of the production and consumption of vegetable tanning materials in the United States. The report covering this survey was prepared under the direction of Wilbur J. Page<sup>7</sup> and the contents are described in detail in this chapter. The world-wide importance of this report can be appreciated when it is realized that the capacity of the United States to produce leather is almost, if not quite, equal to that of the rest of the world.

A list of quantities of the more important vegetable tanning materials consumed in the United States during the year 1922 is given in Table XXXI. The quantities are also calculated in terms of a material containing 12 per cent tannin, as indicated by the official method of the American Leather Chemists' Association, so as to reduce them to a comparative basis and to indicate the concentration of tannin where the materials were previously extracted and sold as concentrated extract.

### Quebracho.

The *Quebrachia lorentzii*, or quebracho tree, furnishes more tannin to the leather world than any other tree or plant. Its wood is very

rich in tannin of high purity. The name quebracho comes from the Portuguese and means "break ax," the wood being so hard that it will turn the edge of a good ax. According to Durland,<sup>3</sup> the quebracho forests of northern Argentina and southern Paraguay have the distinction of being the only forests of their kind in existence. Practically all of the world's supply of this valuable wood comes from this region. Being the hardest, heaviest and most durable wood known, it is used for many purposes other than as a source of tannin. Durland

TABLE XXXI

VEGETABLE TANNING MATERIALS CONSUMED IN THE UNITED STATES DURING THE YEAR 1922

Material	Source	Actual Tons of 2000 Lbs.	Calculated to Tons Material of 12 Percent Tannin
Solid quebracho wood extract . . . .	foreign	43,610	236,221
Liquid " " " . . . .	"	37,508	109,398
Liquid chestnut wood extract . . . .	domestic	118,960	247,833
Powdered " " " . . . .	"	18,665	93,325
Raw hemlock bark . . . . .	"	185,019	185,019
Liquid hemlock bark extract . . . .	"	3,404	7,092
Powdered " " " . . . .	"	393	1,801
Raw oak bark . . . . .	"	148,474	148,474
Liquid oak bark extract . . . . .	"	9,134	19,029
Raw wattle bark . . . . .	foreign	11,604	33,485
Solid wattle bark extract . . . . .	"	725	3,625
Raw myrobalans . . . . .	"	10,297	25,743
Myrobalans extract . . . . .	"	79	329
Raw divi-divi pods . . . . .	"	3,774	12,580
Raw mangrove bark . . . . .	"	4,039	13,463
Mangrove bark extract . . . . .	"	16	73
Liquid spruce bark extract . . . . .	domestic	5,368	11,183
Powdered " " " . . . .	"	102	468
Raw sumac leaves . . . . .	foreign	4,601	10,736
Liquid sumac extract . . . . .	"	268	581
Valonia cups and beards . . . . .	"	7,553	10,297
Solid valonia extract . . . . .	"	58	290
Gambier extract . . . . .	"	55	229
Raw fir bark . . . . .	domestic	101	93
Powdered blended extracts . . . . .	"	308	1,412
Liquid blended extracts . . . . .	"	86	179
Total . . . . .		614,201	1,172,958

estimates the yearly consumption of quebracho wood for all purposes at about one million tons and that it will take about 150 years to exhaust the supply. The possibilities for reforestation are good; a tree 40 years old will furnish about 200 kilograms of heartwood. Extract plants have been erected in Argentina and elsewhere to extract the tannin from the wood and the material appears on the market as concentrated extract. The liquid extract usually contains more than

50 per cent of water and the solid extract less than 25 per cent, the only difference being the extent to which the extract has been dried.

### American Chestnut.

In the United States, one of the most important sources of tannin for heavy leathers, such as sole, belting, and harness, is the American chestnut tree, *Castanea dentata*. This tree is very abundant in Virginia, North Carolina, Tennessee, and northern Georgia. Only the parts of the wood unsuited for lumber are used in the manufacture of chestnut wood extract. The consumption of wood for tanning purposes is more than offset by the yearly growth of the wood. The chestnut tree is able to reproduce itself, by sprouting from the stump, within a period of twenty years. Frey and Leinbach<sup>4</sup> have made a series of analyses of the tannin content at many different points of the chestnut tree; some of their results are included in Table XXX. Unfortunately this valuable tree is menaced by a fungus importation from the orient, called Chestnut Blight. Since 1904, it is reported to have killed 80 per cent of the available chestnut trees north of Virginia and it has penetrated to the vital centers of the remaining stands of chestnut timber. It is to be hoped that this blight will be so effectively stamped out that heavy leather tanners may continue to look to chestnut wood as an inexhaustible supply of tannin.

### Hemlock.

The bark of the hemlock tree (*Tsuga canadensis*) once furnished many American tanneries with their only source of tannin. The tanners used to grind and leach their own bark for use in the tan yard. Hemlock infusions are very astringent and yield a plump and tough leather. Before concentrated extracts were so readily available, it was very desirable for tanneries to locate close to large supplies of bark and many tanneries in northern and eastern United States are monuments to the once great hemlock forests near by.

### Oak.

Like hemlock, oak bark once furnished many tanners with their only source of tannin. The common species used for tanning are the common oak (*Quercus robur*), the tanbark oak (*Quercus densiflora*), and the chestnut oak (*Quercus prinus*). Hemlock imparts a characteristic red color to leather, while oak gives it more of a light tan color, which is often preferred. The increasing scarcity of both hemlock

and oak barks has resulted in the use of mixtures of these barks or their extracts with other concentrated extracts.

Oak wood is poorer in tannin than the bark, so much so, that tanners seldom found it economical to use the wood, but extract manufacturers equipped to concentrate their extracts have utilized it to make commercial grades of oak wood extracts, which have found a place in the tanning of heavy leathers.

#### Wattle.

Various species of *Acacia*, generally known as wattle, have barks that are very rich in tannin of high purity. It was found in Australia and was later introduced into South Africa, from which we now get extensive supplies of the bark.

#### Myrobalans.

The dried nuts of the *Terminalia chebula*, of India, called myrobalans, are rich in tannin and in highly fermentable sugars. The use of myrobalans is much favored in heavy leather yards where it is desired to have acetic acid formed gradually by fermentation. It is not favored for colored upper leathers because it deposits ellagic acid on the surface of the leather. This deposit is called "bloom."

#### Divi-divi.

The pods of the divi-divi (*Caesalpinea coriaria*), of Central America and Brazil, serve a purpose similar to that of myrobalans. They are rich in tannin and form acids very readily by fermentation. They also deposit bloom.

#### Valonia.

Another material rich in tannin and forming an abundance of acid and bloom is valonia, which consists of the cups and beards of the acorns of Turkish oak, *Quercus aegilops*.

#### Gambier.

One of the mildest tanning materials known is gambier extract from the leaves and twigs of the *Nauclea gambir*, of the East Indies. The trees grow to a height of about 10 feet. The twigs and leaves are boiled in water, the extract is strained and thickened with rice meal. For some grades, the paste is baled and for others it is cut into cubes and dried, when it is known as cube gambier. Its use was considered



essential for very fine grades of upper leather until chemists learned that its characteristic mildness was due to certain nontannin constituents that could easily be duplicated in cheaper and more abundant materials.

#### Sumac.

Another very mild tanning material much used for the finer grades of upper leather in the final bath before coloring is Sicilian sumac (*Rhus coriaria*). The dried leaves are leached with water just before using, just as tea is prepared. The material is frequently used to tan sheep skivers for hat bands and as a mordant for chrome tanned leathers to be colored.

#### Leaching.

It is still common to find tanneries equipped to extract the tannin from the raw materials grown in neighboring districts, although the manufacture of tanning extracts has now become a separate industry, which has proved useful in making a greater variety of materials available to the individual tannery.

One of the oldest systems for leaching raw materials, and the one most commonly used in tanneries, is known as the open vat method. The bark, or other material, is broken into small pieces and then shredded in a bark mill. The leaching tanks are usually arranged in batteries of about eight and are fitted with perforated false bottoms on which the bark is placed. The bottom of each tank is fitted with a pipe through which liquor may be drawn off or pumped from one tank to another. When fresh bark is put into a given tank, liquor is run onto it which has been used to leach the bark in all of the seven other tanks. This strong liquor is finally drawn off and pumped into a storage tank. The bark is then leached with liquor which has passed through only six other tanks. The eighth leaching of this bark is made with fresh water, after which the bark is dumped and discarded.

Fresh water is used to leach only the most nearly exhausted bark. As the liquor becomes stronger in tannin, it is run onto fresher bark, and finally onto the previously unleached bark. As soon as each tank is dumped, it is again filled with fresh bark and becomes the head vat in the cycle, which is continuous. The object of this system of leaching is to get final liquors as concentrated as possible. In the tannery, the liquor in the storage tank is used as needed, but in the extract plant it is necessary to evaporate off most of the water so as to make its subsequent transportation practical.

The extraction of the raw material is often facilitated by the use

of mechanical devices. Sometimes the leaching tanks are equipped with mechanical stirrers or with pipes for bubbling air up through the liquor. In another system, the tanks are replaced by revolving drums, used on the same principle as the open vats, the liquor being pumped from one drum to another. In still another system, the bark, or other material, is forced through a trough in one direction, by means of a screw conveyor, while water flows over the bark in the opposite direction. At the point of entry of the fresh water, the bark is practically exhausted and is dumped onto a pile from which it is subsequently moved to the furnaces for fuel, or is disposed of in some other way. At the point of entry of the bark, the liquor is richest in tannin and is conducted to the storage tank.

#### **Effect of Temperature.**

The rate at which tannin can be extracted from the raw material increases with the temperature of the water used, but so also does the rate at which the dissolved matter decomposes. The variation of the ratio of these two rates with temperature determines the optimum temperature that it is desirable to employ and this is different for different materials. It is customary to extract the fresh material at a low temperature and to increase the temperature of extraction until the material is practically exhausted. In using the open vat system for ordinary barks, it is a good plan to have the fresh water at the boiling point and to allow its temperature to fall slowly to about 60° C. as it passes over fresher bark. The temperature of the liquors can be controlled by having suitable heating coils placed in the tanks just under the false bottoms.

#### **Effect of Hardness and Alkalinity of the Water.**

When a very hard, alkaline water is used in leaching, the tannin yield is sometimes low and the extract dark in color and of poor quality. This has been the subject of numerous investigations, from which the general conclusion has been drawn that the use of a soft water in leaching is imperative. But the recent work of Wilson and Kern seems to indicate that the question of hardness of the water used is of less importance than the pH value of water and liquor.

#### **Effect of pH Value on the Color of Tan Liquors.**

Wilson and Kern<sup>9</sup> made a special study of the effect of pH value on the color of gambier and quebracho liquors. Two tan liquors were

prepared, one from gambier and the other from quebracho extract. To each was added sufficient phosphoric acid to bring the pH value to 2.5, as determined by the hydrogen electrode. The phosphoric acid was added to act as a buffer in preventing large changes in pH value upon long standing. To equal portions of each, sodium hydroxide was added to give series of tan liquors ranging in pH value from 3.0 to 12.0 and all having a tannin content of 1 per cent, as determined by the Wilson-Kern method, to be described in the next chapter. The gambier series varied in color from light straw at 3.0 to a very deep red at 12.0. The quebracho series was similar in color excepting that the liquors of lower pH value had a touch of violet. Either series suggested a standard series of colors such as is used in the indicator method of determining hydrogen-ion concentration, except for the fact that a light precipitate formed in all liquors having a pH value of 4.0 or less. The difference in color was evidently a true indicator effect, for any member of one series could be made to match any other member simply by bringing it to the same pH value. All members of either series appeared practically identical when brought to a pH value of 3.0. This complete reversibility of color change, however, was not found when liquors at higher pH values were allowed to stand long exposed to air.

### Effect of pH Value on the Oxidation of Tan Liquors.

Two complete series of each extract were poured into test tubes; the tubes of one series of each were tightly stoppered, while the others were left open to the air. Next day the liquors in the stoppered tubes showed practically no change, but the others had become darker in color, the more so the higher the pH value. When the liquors in a series not exposed to air were all brought to a pH value of 3.0, they all assumed practically the same color. But when those of a series that had been exposed to air were all brought to 3.0, they did not assume the same color, but were darker the higher the pH value during the period of exposure to air; furthermore a precipitate settled out from those whose pH values had been in the vicinity of 9.

This precipitate formation is very curious. A complete series of each extract was allowed to stand exposed to air in shallow dishes for 3 days; the liquors were then made up to original volume and poured into 100-cubic centimeter graduate cylinders. Each was brought to a pH value of 3.0 by the addition of hydrochloric acid and allowed to stand over night. Next day the volume of precipitate from 100 cubic

centimeters of original liquor was read from each cylinder. The results are shown in Fig. 85.

Keeping a solution of either extract exposed to air while its pH value is 9 causes it to yield an enormous precipitate when its pH value is subsequently brought to 3.0. But keeping it exposed to air when its pH value is greater than 10 apparently prevents its precipitation when brought to 3; all such liquors remained brilliantly clear. The addition of a great excess of acid, however, caused all liquors to precipitate, while any precipitate could be completely redissolved by the addition of sufficient alkali.

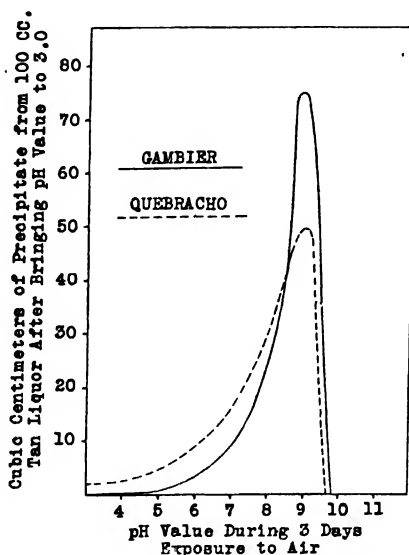


Fig. 85.—Showing how tendency of a tan liquor to form a precipitate when brought to a pH value of 3 varies with its pH value during a period of exposure to air.

Another interesting fact is that the liquors exposed to air when their pH values lay between 8 and 9 gave much trouble with the hydrogen electrode. After bubbling hydrogen through them for only a few minutes, the voltage would fall rapidly towards zero. Even when brought to a pH value of 3.0, the liquors still gave this trouble, making it necessary to check the results by means of indicators. No such trouble was encountered with liquors exposed to air at pH values below 7 or above 10. Apparently  $\text{pH} = 9$  is a critical point in the oxidation of tan liquors.

The curves in Fig. 85 show that this effect of oxidation is ap-

preciable at all pH values from 6 to about 10. Most hard waters have pH values lying within this range and many of them have pH values higher than 8.

### Effect of pH Value on the Precipitation of Tan Liquors.

Wilson and Kern<sup>10</sup> also studied the effect of pH value on the precipitation of quebracho liquors. Four series of solutions of solid quebracho extract were prepared according to the official method of the American Leather Chemists Association, except for the additions of sulfuric acid, hydrochloric acid, sodium hydroxide, and calcium

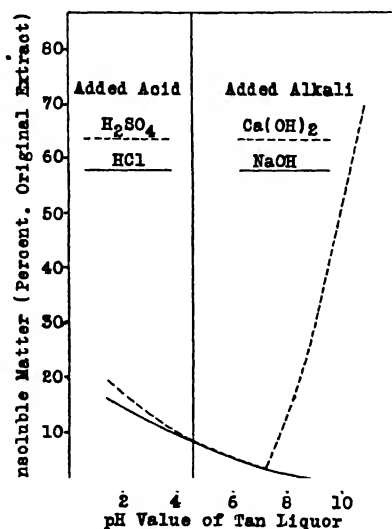


FIG. 86.—Effect of pH value on per cent of insoluble matter in solution of quebracho extract.

hydroxide, respectively, to the four series to produce approximately the desired pH value before making each solution up to the required volume. The pH values were finally determined at 20° C. by means of the hydrogen electrode and the solutions were analyzed according to the official method. The effect of the added acid or alkali upon the per cent of insoluble matter found is shown in Fig. 86.

The solution receiving no addition of acid or alkali had a pH value of 4.60. As the pH value was lowered from this, by the addition of either sulfuric or hydrochloric acid, there was an increase in the per cent of insoluble matter found, sulfuric acid proving the more effective in causing precipitation. With increasing pH value, there was first

a decrease in the amount of insoluble matter and the unfiltered solution gradually became more nearly transparent. In the case of the liquors containing sodium hydroxide, this continued without a break, the liquor having a pH value of 11.35 being quite transparent. But at the neutral point, an abrupt change occurred in the solutions containing calcium hydroxide; with further rise in pH value, the tannin was precipitated in increasing amounts.

If these data may be applied quantitatively to raw tanning materials in general, it is evident that the precipitation of tannin by lime may be prevented by keeping the pH value of water and liquor, during extraction, under 7. But to avoid appreciable oxidation effects, the material should not be extracted at pH values greater than 5, which may be accepted tentatively as the optimum pH value for leaching, since, with decreasing values, there is an increasing amount of material precipitated. Where only hard water is available for leaching, it would seem the part of wisdom to add to it, before using, a sufficient quantity of acid to lower its pH value to 5.

### **Manufacture of Extracts.**

With increasing scarcity of raw tanning materials in the immediate vicinity of tanneries and the need for purchasing tanning materials grown in more remote parts, it has become more profitable for the tanner to purchase concentrated extracts manufactured near the source of the material. Besides effecting a saving in transportation charges, the tanner is freed from the task of doing his own leaching and he usually finds the concentrated extract better suited to his needs than the dilute liquors of the tannery leaching plant. An extract industry has sprung up which has gradually assumed a position of considerable importance.

The first operation consists in grinding the bark to a degree of fineness that will permit ready leaching. It is then put into specially designed tanks and leached after the manner of the open-vat method described above, but with autoclaves instead of open vats. For certain materials there appears to be a fixed condition of temperature and pressure required to get optimum yield and purity. For some materials the leaching is done under a vacuum, the advantage claimed being that an improved quality of extract is obtained. In either the pressure or vacuum systems, the liquor is pumped from tank to tank, as in the open-vat system, fresh water being run onto the nearly exhausted raw material and the most concentrated liquor onto the fresh raw material.

### Clarifying, Decolorizing and Drying.

Some extracts are made muddy or turbid in appearance by the presence of suspended insoluble matter. \*These are clarified before evaporating in a number of different ways, including filter-pressing, centrifuging, and settling and decanting. Sometimes the liquor is treated with blood albumin and then heated to 70° C., at which temperature the albumin coagulates and carries down with it the suspended matters and some of the deeply colored bodies and a little tannin. The clear liquor is decanted off and the sludge is filter-pressed to recover most of the adhering liquor. The slight loss of tannin is more than compensated by the greatly improved color and clarity of the liquor.

A number of other methods of decolorizing involve the treatment of the tan liquor with chemicals. Sulfur dioxide and sodium bisulfite are often used. Some brightening of the color would naturally be expected from the lowering of the pH value of the liquor by sulfur dioxide, but the total effect seems to be more complex than this, since some of the suspended and difficultly soluble matters are thereby rendered soluble. Apparently the reducing action of sulfur dioxide plays a part.

There are naturally numerous methods in use for drying extracts. Since high temperatures and contact with the air during drying are undesirable, much of the drying is done in specially constructed vacuum dryers. As these have been greatly improved, from time to time, it has become possible to dry extracts to greater extents without causing them to suffer any damage. Formerly it was customary to reduce the water content of most extracts only to from 50 to 60 per cent, but now it is not uncommon to find extracts on the market having a water content as low as 10 per cent.

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## Chapter 13.

### Evaluation of Materials Containing Tannin.

For more than a century, chemists have been struggling to perfect a method for measuring the true tanning value of vegetable tanning materials. These materials contain many bodies of unknown chemical composition, which interfere with any attempts to isolate the true tannins, and the compositions of many of the tannins themselves are not known with any degree of certainty. Tanners placed a rough valuation on different materials by noting the amount of leather that could be tanned with a given weight of the material. Realizing the impracticability of trying to measure the individual chemical substances that might be classed as tannins, chemists turned their attention to methods for measuring the constituents of vegetable materials, as a whole, that combine with hide substance to form leather.

It was noted that the soluble constituents of vegetable materials that form stable compounds with hide substance also precipitate gelatin from solution. Thus, in a practical manner, tannin came to be defined as that portion of the water-soluble matter of certain vegetable materials which precipitates gelatin from solution and which can be so completely removed from solution by hide substance that the residual liquor will not precipitate gelatin from solution.

On the basis of this definition, a method was gradually developed in which a solution of the tanning material is shaken with hide powder until it no longer contains any material precipitable by gelatin. The decrease in concentration of dissolved matter effected by the introduction of the hide powder is taken as a measure of the tannin content, it being assumed that only tannin is removed from solution by the hide powder. This is, of course, a fallacy that is generally recognized in the modifications of the method that have been made official by the various associations throughout the world. Even though all tannin is removed from solution by the hide powder, as indicated by the gelatin-precipitation test, the value obtained for tannin varies with the proportion of hide powder to tannin solution, time of contact, and other factors that can be varied at will. In order to get a method



that will give concordant results in the hands of different analysts, it has been found necessary to set arbitrary limits for all factors that might be varied. The weakness of the method lies in these arbitrary limits, which are appreciated, but chemists are apparently not yet agreed as to the best method of eliminating them. This will be discussed in detail later.

There is a movement on foot to get all countries in the world to agree upon one official method for evaluating tanning materials, but at present there are several methods, similar in principle, but differing sufficiently in detail to give appreciably different results. In this chapter two methods will be given completely. They are the official method of the American Leather Chemists' Association and the Provisional International Method.

## Official Method of the American Leather Chemists' Association.

### I. RAW AND SPENT MATERIALS.

#### (1) *Caution:*

Proper care must be taken to prevent any change in the water content of raw materials during the sampling and preliminary operations. (See "General" under Sampling.)

#### (2) *Preparation of Sample:*

The sample must be ground to such a degree of fineness that the entire sample will pass through a sieve of 20 meshes to the inch (linear).

(a) The temperature used for drying samples of spent material for grinding must not exceed 60° C.

(b) Samples of raw material too wet to be ground may be dried before grinding as in (a). In this case a preliminary water determination must be made according to (IV) on the sample as received. If the portion of the sample taken for the water determination is in pieces too large to dry properly, it is permissible to reduce these to smaller size as rapidly and with as little loss of water as possible.

#### (3) *Water Determination:*

Ten grams of the ground material shall be dried in the manner and for the period specified for evaporation and drying in extract analysis (see IV).

#### (4) *Amount of Sample to be Extracted:*

Such an amount of raw material shall be extracted as will give a solution containing as nearly as practicable 0.4 gram tannin to 100 cc. (not less than 0.375 or more than 0.425). Of spent materials such an amount shall be taken as will give a solution of as nearly as practicable the above concentration.

#### (5) *Extraction:*

Extraction shall be conducted in an apparatus consisting of a vessel in which water may be boiled and a container for the material to be extracted. The container shall be provided above with a condensation chamber so arranged that the water formed from the condensed steam will drip on the material to be extracted, and provided below with an arrangement of outlets such that the percolate may either be removed from the apparatus or be delivered to the boiling vessel. The boiling vessel must be so connected that it will deliver steam to the condensation chamber and that it may receive the percolate from the container. The condensation water from the condenser must be at approxi-

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mately the boiling temperature when it comes in contact with the material to be extracted.

The material of which the boiling flask is composed must be inert to the extractive solution. Suitable provisions must be made for preventing any of the solid particles of the material from passing into the percolate.

(A) Woods, Barks and Spent Materials:

Five hundred cc. of the percolate shall be collected outside in approximately 2 hours and the extractions continued with 500 cc. for 14 hours longer by the process of continuous extraction with reflux condenser. The applied heat shall be such as to give condensation approximating 500 cc. in  $1\frac{1}{2}$  hours.

(B) Materials Other than Woods, Bark and Spent:

Digest the material in the extractor for 1 hour with water at room temperature and then extract by collecting 2 liters of percolate outside in approximately 7 hours.

(6) *Analysis:*

The percolate shall be heated to 80° C., cooled, made to the mark and analyzed according to the official method of extracts.

### II. ANALYSIS OF EXTRACT.

(7) *Amount and Dilution for Analysis:*

(A) Fluid Extracts:

Fluid extracts shall be allowed to come to room temperature, be thoroughly mixed, and such quantity weighed for analysis as will give a solution containing as nearly as possible 0.4 gram tannin to 100 cc. (not less than 0.375 nor more than 0.425). Precautions must be taken to prevent loss of moisture during weighing. Dissolve the extract by washing it into a liter flask with 900 cc. of distilled water at 85° C.

Cooling:

(a) The solutions prepared as above shall be cooled rapidly to 20° C. with water at a temperature of not less than 19° C., be made to the mark with water at 20° C. and the analysis proceeded with at once, or

(b) The solution shall be allowed to stand over night, the temperature of the solution not being permitted to go below 20° C., be brought to 20° C. with water at not less than 19° C., be made to the mark with water at 20° C. and the analysis proceeded with.

(B) Solid and Powdered Extracts:

Such an amount of solid or powdered extract as will give a solution of the strength called for under liquid extracts shall be weighed in a beaker with proper precautions to prevent change of moisture. One hundred cc. of distilled water at 85° C. shall be added to the extract and the mixture placed on the water-bath, heated and stirred until a homogeneous solution is obtained. When dissolved, the solution shall immediately be washed into a liter flask with 800 cc. of distilled water at 85° C., be cooled, etc., as under (A) above.

NOTE—It is permissible to make up 2-liter instead of 1-liter solutions dissolving by washing into flask with 1,800 cc. water at 85° C. in case of fluid extracts and 1,700 cc. water at 85° C. in case of solid or powdered extracts.

(8) *Total Solids:*

Thoroughly mix the solutions; pipette 100 cc. into tared dish, evaporate and dry as directed under "Evaporation and Drying." (See IV.)

(9) *Water:*

The water content is shown by the difference between 100 per cent and the total solids.

(10) *Soluble Solids:*

S. & S. No. 590, or Munktell's No. 1F, 15 cm. single, pleated, filter paper shall be used for the filtration.

The kaolin used shall answer the following test: 2 grams kaolin digested with 200 cc. of distilled water at 20° C. for 1 hour shall not give more than 1 mg.

of soluble solids per 100 cc., and shall be neutral to phenolphthalein. To 1 gram kaolin in a beaker add sufficient solution to fill the paper, stir and pour on paper. Return filtrate to paper when approximately 25 cc. has collected, repeating operation for 1 hour, being careful to transfer all kaolin to the paper. At the end of the hour remove solution from filter paper, disturbing the kaolin as little as possible. Bring so much as needed of the original solution to exactly 20° C. as described under (7), refill the paper with this solution and begin to collect the filtrate for evaporating and drying so soon as it comes clear. The paper must be kept full and the temperature of the solution on the filter must not fall below 20° C. nor rise above 25° C. during this part of the filtration. The temperature of the solution used for refilling the paper must be kept uniformly at 20° C. and the funnels and receiving vessels must be kept covered.

Pipette 100 cc. of clear filtrate into tared dish; evaporate and dry as under IV.

#### (11) Insolubles:

The insoluble content is shown by the difference between the total solids and the soluble solids, and represents the matters insoluble in a solution of the concentration used under the temperature conditions prescribed.

#### (12) Non-Tannins:

The hide powder used for the non-tannin determination shall be of woolly texture, well delimed, and shall require between 12 and 13 cc. of N/10 NaOH to neutralize 10 grams of the absolutely dry powder.

(a) Digest the hide powder with 10 times its weight of distilled water till thoroughly soaked. Add 3 per cent of chrome alum,  $\text{Cr}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , in 3 per cent solution calculated on the weight of the air-dry powder. Agitate frequently for several hours and let stand over night. Squeeze and wash by digesting with 4 successive portions of distilled water, each portion equal in amount to 15 times the weight of the air-dry powder taken. Each digestion shall last for 15 minutes, and the hide powder shall be squeezed to approximately 75 per cent water after each digestion except the last, a press being used if necessary. The wet hide powder used for the analysis shall contain as nearly as possible 73 per cent of water, not less than 71 per cent nor more than 74 per cent. Determine the moisture in the wet hide powder by drying approximately 20 grams. (See IV.) To such quantity of the wet hide as represents as closely as practicable 12½ grams (not less than 12.2 nor more than 12.8) of absolutely dry hide add 200 cc. of the original analysis solution and shake immediately for 10 minutes in some form of mechanical shaker. Squeeze immediately through linen, add 2 grams of kaolin (answering test described under (9) to the detannized solution and filter through single folded filter (No. 1F Swedish recommended) of size sufficient to hold the entire filtrate, returning until clear. Pipette 100 cc. of filtrate into tared dish, evaporate and dry as under IV.

The weight of the non-tannin residue must be corrected for the dilution caused by the water contained in the wet hide powder.

Funnel and receiving vessels must be kept covered during filtration. Flasks graduated to deliver 200 cc. are recommended for measuring the analysis solution to be detannized.

**Norm.**—In order to limit the amount of dried hide powder used, determine the moisture in the air-dry powder and calculate the quantity equal to 12½ grams of actual dry hide powder. Take any multiple of this quantity according to the number of analyses to be made, and after chroming and washing as directed, squeeze to a weight representing as nearly as possible 73 per cent of water. Weigh the whole amount and divide by the multiple of the 12½ grams of actual dry hide powder taken to obtain the weight of wet hide powder for 200 cc. of solution.

#### (13) Tannin:

The tannin content is shown by the difference between the soluble solids and the corrected non-tannins, and represents the matters absorbable by hide under the conditions of the prescribed methods.

## III. ANALYSIS OF LIQUOR.

(14) *Dilution:*

Liquors shall be diluted for analysis with water at room temperature so as to give as nearly as possible 0.7 gram solids per 100 cc. of solution. Should a liquor be of such character as not to give a proper solution with water of room temperature it is permissible to dilute with water at 80° C. and cool rapidly as described under (7, A, a).

(15) *Total Solids:*

To be determined as in Extract Analysis.

(16) *Soluble Solids:*

To be determined as in Extract Analysis.

(17) *Insolubles:*

Determined as in Extract Analysis.

(18) *Non-Tannins:*

To be determined by shaking 200 cc. of solution with an amount of wet chromed hide powder, containing as nearly as possible 73 percent water, corresponding to an amount of dry hide powder shown in the following table:

Tannin range per 100 cc.	Dry powder per 200 cc.
0.35—0.45 gram	9.0—11.0 grams
0.25—0.35 gram	6.5—9.0 grams
0.15—0.25 gram	4.0—6.5 grams
0.00—0.15 gram	0.0—4.0 grams

Solutions to be shaken for non-tannins as in Extract Analysis and 100 cc. evaporated as in Extract Analysis.

## IV. TEMPERATURE, EVAPORATION AND DRYING, DISHES.

(19) *Temperature:*

The temperature of the several portions of each solution pipetted for evaporating and drying, that is, the total solids, soluble solids and non-tannins must be identical at the time of pipetting.

(20) *Evaporation:*

All evaporation and drying shall be conducted in the form of apparatus known as the "Combined Evaporator and Dryer" at a temperature not less than 98° C. The time for evaporation and drying shall be 16 hours.

(21) *Dishes:*

The dishes used for evaporation and drying of all residues shall be flat-bottomed glass dishes of not less than 2¼ inches diameter nor more than 3 inches in diameter.

## V. DETERMINATION OF TOTAL ACIDITY OF LIQUORS.

(22) *Reagents:*

(a) One per cent solution of gelatin neutral to hematine. The addition of 25 cc. of 95 per cent alcohol per liter is recommended to prevent frothing. If the gelatin solution is alkaline, neutralize with tenth normal acetic acid and if acid neutralize with tenth normal sodium hydroxide.

(b) Hematine. A solution made by digesting hematine in cold neutral 95 per cent alcohol in the proportion of ½ gram of the former to 100 cc. of the latter.

(c) Acid washed kaolin free from soluble matters.

(d) Tenth normal sodium hydroxide.

*Directions:*

To 25 cc. of liquor in a cylinder that can be stoppered, add 50 cc. of gelatine solution, dilute with water to 250 cc., add 15 grams of kaolin and shake vigorously. Allow to settle for at least 15 minutes, remove 30 cc. of the supernatant solution, dilute with 50 cc. of water and titrate with tenth normal soda using hematine solution as the indicator. Each cc. tenth normal soda is equivalent to 0.2 per cent acid as acetic.

## VI. GENERAL.

(23) When materials containing sulfite-cellulose extract are analyzed, the fact that the material contains sulfite-cellulose extract shall be noted on the report.

(24) The test for the presence of sulfite-cellulose in a liquor or extract shall be as follows: 5 cc. of a solution of analytical strength shall be placed in a test-tube, 0.5 cc. of aniline added and the whole well shaken; then 2 cc. of strong hydrochloric acid added and the mixture again shaken. If at least as much precipitate remains as is obtained when a comparison solution prepared as below is similarly treated, the material shall be held to contain sulfite-cellulose.

The comparison solution shall consist of sulfite-cellulose in the proportion of one part total solids to 2,000 cc. of solution, and as much tanning material, similar to that being tested, but known to be free from sulfite-cellulose, as will make up the solution to analytical strength. Attention is drawn to the fact that certain synthetic tannins give precipitates under the conditions of this method.

(25) On public analytical work by members of this Association the fact that the Official Method has been used, shall be so stated.

## OFFICIAL METHOD FOR SAMPLING TANNING MATERIALS.

*General:*

Extract whether liquid or solid, and tanning materials in general all contain moisture. The amount of moisture varies with climatic conditions, but especially in liquid, and in most solid extracts becomes less as the extract is exposed to the air. As the value of any material shown by analysis is directly dependent upon the amount of moisture contained, and as an exposure of a comparatively few moments may alter appreciably the amount of moisture it is apparent that the sampling in all its details should be done as quickly as consistent with thoroughness and with great care to expose the material as little as possible to the air. The portions taken as samples should be placed at once in containers as nearly air tight as possible, and preferably of glass. Wood, cardboard, poorly glazed crockery, etc., are all porous and more or less absorbent and not suitable for retaining samples.

Liquid extract cannot be accurately sampled when it contains any frozen material. A sample of extract taken after live steam has been run into the extract has not the same concentration as the original extract. A sample of spent bark which has been standing where dust from fresh ground bark has sifted into it does not represent the degree of extraction of the spent bark. Samples of liquor which have been kept with no preservative in them for some time do not represent the condition of the liquor when sampled.

All extracts and crude tanning materials shall be sampled as nearly as possible at time of weighing, and for every 50,000 pounds, or less, sampled a sample shall be drawn.

*(1) Solid, Powdered and Pasty Extracts:*

The number of packages to be sampled out of a given lot shall be ascertained by taking a percentage of the total number of packages in the lot obtained in the following manner: Divide the total number of packages by 100, multiply by 0.02 and subtract from 4.

Thus

$$\begin{aligned} 4,700 \div 100 &= 47 \\ 47 \times 0.02 &= 0.94 \\ 4 - 0.94 &= 3.06 \text{ per cent} \\ 4,700 \times 0.0306 &= 144 \text{ packages.} \end{aligned}$$

Provided that for lots of 200 packages and under 5 per cent of the number of packages shall be sampled, and for lots of 10,000 packages and over 2 per cent of the number of packages shall be sampled.

Whenever possible every Nth package shall be set aside for sampling while the extract is being moved. When this is not possible, the packages shall be selected from as uniformly distributed parts of the bulk as possible.

Samples of as nearly equal size as practicable shall be taken from each package and these samples shall represent as nearly as may be, proportionally the outer and inner portions of the extract. These sub-samples shall be placed in a clean, dry closed container. When sampling is completed, the whole composite sample shall be broken up until it will pass through a sieve of 1-inch mesh; it shall be reduced to the required bulk by successive mixings and quarterings. From this bulk duplicate samples of at least 6 ounces shall be drawn from opposite quarters by means of a small flat scoop (and not by selecting a handful here and there). The sample shall be enclosed in the smallest clean, dry, glass receptacle, sealed and properly labeled.

NOTE.—Whenever possible the sample should be wrapped in paraffine paper and placed in the smallest straight-side glass receptacle; especially is this desirable during the warmer months of the year.

Sampling at place of manufacture shall be conducted by running a portion from the middle of each strike into a mold holding at least 2 pounds. These sub-samples shall be preserved with proper precautions against evaporation, and be sampled for analysis as above.

### (2) *Liquid Extracts in Barrels:*

The number of barrels of extracts to be sampled out of any given lot shall be not less than 10 per cent of the whole number of barrels for every 50,000 pounds or fraction thereof. The barrels to be sampled shall be rolled and shaken from end to end until the contents are homogeneous. Whenever this is not possible the heads of the barrels shall be removed and the contents stirred until homogeneous, a sample of equal size to be taken from each barrel. These sub-samples shall be put together in a suitable closed container and be thoroughly mixed. From this bulk duplicate samples of at least 4 ounces shall be drawn and preserved in clean, dry, glass containers; sealed and labeled with such distinguishing marks as may be necessary.

### (3) *Liquid Extract in Bulk:*

The extract shall be agitated with air, be plunged or be mixed by some other efficient means until homogeneous. Equal samples shall then be taken from different parts of the bulk, be placed in a proper container, be thoroughly mixed and sampled as described in (2).

### (4) *Liquid Extract in Tank Cars:*

The following methods are permissible:

(a) The extract shall be unloaded into clean, dry containers and sampled according to (3); or,

(b) The extract shall be mixed until homogeneous, by plunging through the dome or other effective means, then numerous equal samples shall be taken from as widely scattered parts of the bulk as possible. These samples shall then be placed in a suitable container, be mixed and sampled as in (2).

NOTE.—As it is almost impossible to secure a homogeneous mixture of the extract in a tank car, this method should be used only when no other is possible. Or,

(c) The extract shall be sampled as follows while the car is being unloaded.—A quart sample shall be taken from the discharge three minutes after the extract has begun to run; another quart sample shall be taken three minutes before the extract has all run out, and three other quart samples shall be taken at equal intervals between these two. These five samples shall be transferred to a suitable container as soon as taken, be thoroughly mixed and sampled as in (2).

**(5) Crude Tanning Materials:**

A. Shipments in bags, mats or other similar packages.

A number of packages shall be sampled representing 2 per cent of the weight for every shipment of 50,000 pounds or fraction thereof, by taking representative portions from each package. These sub-samples shall be mixed together and the bulk be reduced by mixing and quartering to the desired size. Duplicate samples of not less than 5 pounds each shall be preserved in air-tight containers properly labeled.

B. Shipments in bulk, bark, wood, etc., in sticks.

Sticks shall be taken from at least ten uniformly distributed parts of the bulk, be sawed completely through and the sawdust thoroughly mixed and sampled as in "A."

C. Materials prepared for leaching.

Samples of equal size shall be taken at uniform intervals as the material enters the leach and be kept in a suitable container till sampling is completed. This bulk shall then be thoroughly mixed, be reduced by mixing and quartering, and duplicate samples for analyses of at least 2 pounds in size be preserved in air-tight containers, as in "A."

**(6) Spent Materials from Leaches:**

Samples of spent material shall be taken from the top, middle and bottom, and in each case from the center and outer portions of the leach. These sub-samples shall be thoroughly mixed, be reduced in bulk by mixing and quartering, and duplicate samples of at least 1 quart in size be preserved for analysis.

**(7) Tanning Liquors:**

The liquor shall be mixed by plunging or other effective means till homogeneous and then samples of at least 1 pint be taken for analysis. The addition of 0.03 per cent of thymol or other suitable anti-ferment to the sample is essential to keep the liquor from altering its original condition.

When routine samples are taken from day to day and a composite sample analyzed, samples of equal size shall be taken from each vat after thorough mixing, be preserved in covered containers in as cool a place as possible, and be kept from fermentation by the addition of suitable anti-ferment, as above. This bulk shall be mixed till homogeneous and samples of not less than 1 pint each be preserved for analysis.

When a sample is taken by a member of this Association in accordance with the above method, it is requested that he state upon the label of the sample submitted and upon the analysis blank that "this sample has been taken in accordance with the official method of sampling of The American Leather Chemists Association."

## Provisional International Method of Quantitative Tannin Analysis.

### A. GENERAL REGULATIONS.

#### APPARATUS.

##### 1. Glassware.

The glassware employed shall be resistant to the action of distilled water; graduated flasks and pipettes shall be carefully verified, and if necessary, re-graduated. The graduation marks on the 1 and 2 liter flasks must be near the base of the neck.

##### 2. Desiccators.

Desiccators shall possess a tight fitting cover and contain sulfuric acid which shall not fall below a concentration of 85 percent  $\text{H}_2\text{SO}_4$  (by weight). Only one dish shall be in each desiccator at any one time.

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### 3. *Evaporating Basins.*

Evaporating basins must be shallow, flat bottomed and not less than 7 cm. nor more than 8.5 cm. in diameter. Glass, porcelain or silver basins may be used. Glass basins must not be allowed to come in direct contact with steam; porcelain basins must be glazed outside and inside. Porcelain rings must be used on the water bath or steam oven when silver basins are employed.

### 4. *Apparatus for Evaporating and Drying.*

Evaporation must be carried out at 98.5°-100° C. on:—

(a) a water bath

(b) a combined water bath-steam oven, or

(c) a combined evaporator and dryer

After evaporation as in (a) or (b), residues must be dried in an oven at a uniform and constant temperature of 98.5°-100° C.

Water, steam and electric ovens at ordinary pressure or under vacuum are permissible, but electric ovens must be so equipped that they will maintain an essentially constant temperature.

Gas-heated air ovens must not be used.

### 5. *Balances.*

Analytical balances, accurate to at least 0.2 mg. with 100 g. load, shall always be employed for weighing residues.

### 6. *Linen.*

Linen cloths are to be used for washing the chromed hide powder and for the preliminary filtration of the detannized solutions. The linen must be freed from weighting matter by boiling in several changes of distilled water.

### 7. *Filter Paper.*

The filter papers employed shall be pleated and 15 cm. in diameter; they must be used single. Any of the following papers may be used: Munktell No. 1F, S. and S. No. 590, or Durieux "Super."

### 8. *Koch Extractor.*

This apparatus consists of a wide-mouthed glass flask of 300 to 400 cc. capacity, which is thin walled and well annealed so that it will withstand continual heating in a water bath. The mouth of the flask is closed by a rubber stopper through which pass two glass tubes, one through which the water enters ending about 1 cm. below the bottom of the stopper in order to mix the inflowing cold water with the contained hot water, and the other, which is the outlet, going almost to the bottom of the flask, the end being enlarged into a funnel shape and the opening covered with silk gauze. Both tubes are bent at right angles just above the stopper and connected to rubber tubing. On the bottom of the flask is placed a layer of fine sea sand 2 cm. deep (the sand having been purified by washing with HCl and water) and on the sand is placed the properly prepared material to be extracted. To fill the flask, the tube which reaches to the bottom is connected by rubber tubing to a right-angled glass tube, the free end of which dips in a beaker of water; suction is now applied to the second tube until the flask is full, when the rubber tube is then securely closed by means of a pinch cock and the apparatus placed in a water bath. The short inlet tube is next suitably connected to a 2-liter bottle standing about 150 cm. above the water bath as shown in Fig. 87.

## CHEMICALS AND REAGENTS.

### 9. *Distilled Water.*

This must comply with the following specifications:

(a) The pH value must be between 5.0 and 6.0; that is, it must not yield a red color with methyl red nor a deep purple with brom cresol purple (brom cresol sulfonephthalein).

(b) The residue, after evaporation of 100 cc., must not exceed 0.001 gram.



10. *Kaolin.*

Kaolin must be of such quality that when 1 gram is suspended in 100 cc. of water and well shaken, the suspension will have a pH value between 4.0 and 6.0; that is, it will not give a red color with methyl orange nor a deep purple color with brom cresol purple. When 1 gram of the kaolin is shaken with 100 cc. of 0.01N acetic acid and the mixture filtered, the filtrate must leave less than 1 milligram of residue after evaporation and drying.

Kaolin not meeting these specifications may often be made to comply with them by digesting with hydrochloric acid and then washing with distilled water until free from soluble matter.

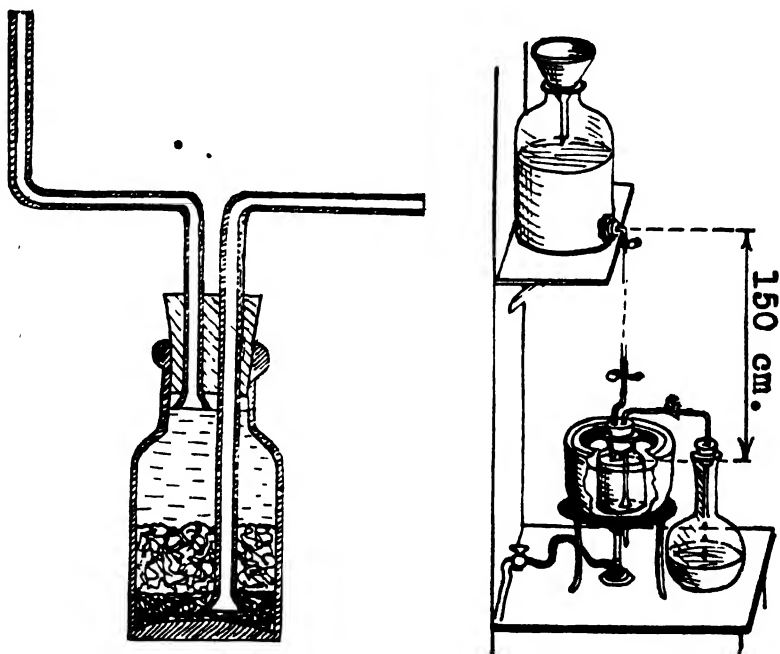


Fig. 87.—Showing arrangement of Koch extractor.

NOTE—The following kinds of kaolin have been found satisfactory: LeMoor China Clay and "Catalpo," but each lot must be tested and shown to conform to the above specifications.

11. *Chrome Alum Solution.*

Chrome alum must be crystalline and correspond in composition to the formula  $\text{Cr}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ . The solution used for chroming hide powder must be prepared at laboratory temperature by dissolving the alum in water in the proportion of 30 grams to 1 liter of water. The chrome alum solution must not be more than 30 days old when used.

12. *Hide Powder.*

The hide powder employed shall have been approved by an International Hide Powder Committee, consisting of the chairmen of the Hide Powder Committees of the A. L. C. A., the I. S. L. T. C., and the I. V. L. I. C. It must comply with the following conditions:

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(a) The ash content must be less than 0.3 per cent.

(b) When 7 gm. of the air-dry powder is suspended in 100 cc. of N/10 potassium chloride solution, left for 24 hours with occasional shaking, and the mixture then filtered through filter paper, the pH of the filtrate must not be less than 5.0 nor more than 5.4.

### 13. *Gelatin Salt Reagent.*

One g. of photographic gelatin and 10 g. pure sodium chloride are dissolved in 100 cc. of distilled water and the reaction adjusted to pH 4.7 approximately by adding acid or alkali, that is, the solution should give a red color with methyl red and a yellow with methyl orange. Two cc. of toluene added to this solution will preserve it for a short time if kept in a cool place, but a freshly made solution is preferable. In the preparation of this solution, the temperature must not be allowed to exceed 60° C.

### PREPARATION OF SAMPLES FOR ANALYSIS.

#### 14. *Solid Tanning Materials (Woods, Barks, Fruits, Etc.).*

Woods, barks and fruits must be ground in a suitable mill until they will pass through a sieve of five wires per linear centimeter. When from the fibrous nature of a solid tanning material it cannot be ground so as to pass entirely through the specified sieve, the finer and coarser portions must be separately weighed so as to determine the proportion of fine and coarse material in the whole amount ground. The quantity of material actually used for extraction must consist of fine and coarse material in this same proportion.

Any material giving finely divided matter (dust) on grinding, must be dealt with in the same way, that is, the portion extracted must consist of dust and coarser material in the proportions existing in the whole ground sample.

Fibrous tanning materials such as leaves (sumac, etc.) and barks (oak, mimosa, mangrove, etc.) may be pounded in a mortar (preferably of copper or bronze with a heavy copper pestle) in order to break up the fibrous matter and to facilitate the penetration of the water used for extraction.

Some materials lose moisture when submitted to the grinding process, and it is advisable therefore to estimate the moisture both before and after grinding, and if any loss has taken place, the results obtained on the dried sample should be calculated back to the original moisture.

Materials that deposit ellagic acid, such as valonia, myrobalans, etc., must be heated to 100° C. for 1 hour before they are extracted.

#### 15. *Solid Extracts.*

Solid extracts shall be ground in a porcelain or agate mortar before weighing for analysis. In case the solid extracts are of uneven moisture content and cannot be pulverized and ground, the blocks should be broken up, a portion weighed in a flat-bottomed basin, allowed to dry in an oven at 70° C. for some hours and then be left exposed to the atmosphere of the laboratory for several hours (preferably overnight). After this partial drying, the extract is weighed and the loss of water calculated. The extract is next finely pulverized in a mortar and a weighed portion is dried in an oven between 98.5 and 100° C. to constant weight. This new loss of moisture is added to the first for calculating the percentage of water in the original sample.

Pasty extracts, such as black gambier, should be cut up into small portions and treated in the same way.

#### 16. *Liquid Extracts.*

Liquid extracts shall be thoroughly mixed so as to insure a perfectly homogeneous sample, care being taken to include any sediment that has settled in the bottom of the container. Viscous extracts shall be heated to 45° C. on a water bath, well mixed, cooled to 18° C. (see para. 21) and then weighed at once. This heating must be mentioned on the report.

17. *Preparation of Infusion.*

Such quantity of material shall be employed as will give a solution containing as nearly as possible 4 grams per liter of tanning matter absorbable by hide powder and in any case not less than 3.75 nor more than 4.25 grams. In the event of the results of an analysis showing a tannin strength outside these limits, the analysis must be repeated on the proper weight of material.

All materials for analysis shall be weighed out on an analytical balance to an accuracy of at least 0.002 gram.

18. *Extraction of Solid (Crude) Tanning Materials.*

Solid tanning materials ground as previously described shall be extracted in a Koch extractor, using such a quantity as to give 2,000 cc. of solution of the required analytical strength. (See para. 17). The material must be soaked in cold distilled water in the extractor (see para. 8) for not less than 12 nor more than 18 hours (*c. g.*, overnight) before commencing extraction.

At the end of this time the infusion must be drawn off and the extraction be continued at such uniform speed that the required 2 liters will be obtained in four hours. When the first 150 cc. have been collected, the temperature of the water bath must be raised to 50° C. and at this temperature an additional 750 cc. collected. The temperature must then be raised rapidly to boiling and the further quantity required to make 2 liters be extracted as near boiling as possible.

Woods and such barks as oak and hemlock must be extracted so that the 2 liters will be secured by uniform extraction during 7 hours instead of 4.

19. *Liquid Extracts.*

Liquid extracts shall be weighed out as rapidly as possible, avoiding change in moisture content, in a stoppered weighing bottle. Dissolve by washing into a 1 liter flask containing approximately 400 cc. distilled water at 85° C. to make the volume finally equal to 900 cc. Then proceed as directed under "Cooling Solutions," (Paragraph 21).

Materials sensitive to water at 85° C. may be dissolved at a lower temperature and this must be stated on the report.

20. *Solid, Powdered or Pasty Extracts.*

Solid, powdered and pasty extracts shall be weighed out in a beaker as rapidly as possible, avoiding change in moisture content. Approximately ten times the weight of the extract, of distilled water shall be added, the mixture placed on a steam bath, heated with frequent stirring until solution or a uniform suspension is obtained. Wash this solution into a 1 liter flask containing approximately 400 cc. of distilled water at 85° C. with enough distilled water at 85° C. to make the volume finally equal to 900 cc. Then proceed as directed under "Cooling of Solutions." (Para. 21).

In the case of extracts containing more than 45 percent tannin, such quantities shall be taken as to yield 2 liters of infusion of analytical strength.

21. *Cooling of Solutions.*

After dissolving extracts or extracting solid tanning materials, the infusions shall then be cooled to 18° C as follows:

Immerse the flask in any large vessel or trough containing water at 18° C. and maintain the cooling water at that temperature during the entire period of cooling. Keep the solution in the flask well agitated throughout the cooling process. This procedure is absolutely necessary to insure uniform results. After cooling, make up the required volume with distilled water, thoroughly mix and proceed with the filtration.

NOTE.—In hot climates where there are difficulties in maintaining this temperature (18° C.), the flasks may with advantage be enclosed in paper bags after cooling.

## B. ANALYSIS.

22. *General Instructions.*

The solutions of total solids, solubles and nontannins must be at the same temperature when pipetted.

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### 23. *Estimation of Moisture and of Total Solids.*

For any tanning extract or material, the sum of the moisture and total solids is 100 percent, so that a determination of either quantity is sufficient. In the case of solid (crude) tanning materials and also of any solid or pasty extracts that do not yield to uniformly turbid solution, a direct determination of moisture must be made.

### 24. *Moisture.*

About 1 gram of the finely ground material is accurately weighed out in a squat-shaped wide-mouthed weighing bottle and dried between 98.5 and 100° C. in a water or steam oven for 3 to 4 hours, cooled in a desiccator for 20 minutes and weighed on an analytical balance as quickly as possible. It is then returned to the oven and the drying continued until constant weight is attained. If any weighing shows an increase in weight over a preceding, the lowest weight found must be taken.

### 25. *Total Solids.*

Total Solids are determined by evaporating to apparent dryness on a water bath or a combined water bath-steam oven, 50 cc. of the well mixed and uniformly turbid tannin infusion, in the evaporating basins previously described. (Para. 3).

The residues in the basins are then promptly dried between 98.5°-100° C. for three to four hours (see para. 4), cooled in desiccators and weighed as rapidly as possible to an accuracy of 0.2 mg. and this repeated until constant weight is attained. The basins must not be wiped after removal from the desiccator.

It is permissible to use the combined evaporator and dryer and to make one operation of the evaporating and drying.

### 26. *Solubles.*

To the amount of the analysis solution required to fill the filter (about 75 cc.) in a beaker, add 1 gram of kaolin, mix thoroughly and pour immediately on to the filter paper (see para. 7). Collect the filtrate in the same beaker and when approximately 25 cc. have been collected, return the filtrate to the paper, repeating this operation for 1 hour, taking care to transfer in this manner all kaolin to the filter. At the end of 1 hour remove the solution on the filter, disturbing the kaolin as little as possible, as for example, by syphoning. Bring as much as needed of the original solution to 18° C., as described under "Cooling of Solutions." (Para. 21.) Refill the filter with this solution and begin to collect the filtrate for pipetting as soon as the filtrate becomes optically clear, discarding so much filtrate as comes through prior to its coming clear. Keep the filter full, the temperature of the filtering solution at 18° C., and the funnel and collecting vessel covered. Pipette 50 cc. of the clear filtrate into a tared basin, evaporate, dry, cool and weigh to constant weight as described above.

The solution is considered to be "optically clear" both by reflected and transmitted light when a bright object such as an electric light filament is distinctly visible through at least 5 cm. thickness, and when a layer 1 cm. deep in a beaker, placed in a good light on black glass or black glazed paper, appears dark and free from opalescence when viewed from above.

### 27. *Preparation of Chromed Hide Powder.*

A multiple of that quantity of hide powder containing 6.25 gm. of dry matter, according to the number of analyses to be made, together with 6 gm. for the moisture determination, is digested with ten times its weight of distilled water for 1 hour. To this is added for each gram of air-dry powder taken, 1 cc. of the stock chrome alum solution (para. 11) and the whole well stirred.

Continue to stir frequently for several hours and then let stand overnight. In the morning transfer the chromed powder to a clean linen filter cloth, drain, and squeeze. Place the cloth containing the powder in a suitable vessel (an enamel bucket is suitable for large quantities), open out the cloth bag fashion, and pour on to the powder a quantity of water equal to 15 times the weight of

the air-dry powder taken. Mix the powder and water thoroughly and digest for 15 minutes, after which lift out the cloth and powder and immediately drain and squeeze to approximately 75 percent moisture, using a press if necessary. Digest the powder three more times in the same manner, using distilled water throughout. At the end of the final digestion, squeeze the powder so that it will contain as nearly as possible 73 percent moisture, not less than 72 or more than 74 percent. (It is convenient to squeeze the powder slightly drier than specified, then transfer it as quantitatively as possible to a tared vessel and carefully add water to give the proper moisture content as determined by weighing). Thoroughly break up the cake of wet, chromed powder and mix until uniform and free from lumps. Weigh at once 20 g. of the wet chromed powder and determine moisture in it as directed in para. 24. Also weigh at once the charges for the non-tannin determinations, transfer them to shake bottles, and stopper the bottles tightly.

#### 28. *Non-Tannin Determination.*

To such quantity of the wet chromed hide powder as represents as nearly as possible 6.25 gms., not less than 6.1 gms. nor more than 6.4 gms. of absolutely dry hide powder, add 100 cc. of the analysis solution and shake immediately for exactly 10 minutes in a mechanical rotary shaker at 50-65 revolutions per minute. Pour powder and solution on a clean, dry linen cloth supported by a funnel, drain and squeeze by hand. Add to the filtrate 1 g. of kaolin that meets the requirements of para. 10, mix thoroughly and pour into a single 15.0 cm. pleated filter paper, returning the filtrate repeatedly until it is clear. Keep the funnel and collecting vessel covered during filtration. (The filtrate must be tested with the gelatin-salt reagent (see para. 13) and if 10 cc. gives any turbidity with 1 or 2 drops of the reagent, the fact must be stated on the report of the analysis). Pipette 50 cc. of the filtrate into a tared dish, evaporate, dry, cool and weigh. Correct the weight of the residue for the dilution caused by the water in the wet hide powder and calculate the percentage of non-tannins.

#### 29. *Tanning Matter Absorbable by Hide Powder.*

This is the difference between the percentages of total solubles and non-tannins.

#### 30. *Insoluble Matter.*

Insoluble matter is the difference between the percentages of total solids and total solubles or between 100 percent and the sum of the percentages of moisture and soluble matter in the cases of those solid tanning materials and solid and pasty extracts in which moisture is directly estimated.

#### 31. *Specific Gravity.*

Specific gravity shall be determined by the specific gravity bottle or pycnometer, keeping the temperature as closely as possible to 15° C.

#### 32. *Accuracy of the Method.*

All analyses shall be the average result of duplicate determinations. The weights of residues shall in all cases agree within 2 mg. so that the absolute error in the tannin content is not more than 2 percent. For example, for liquid extracts containing 30 percent of tannin, the duplicate results for percentage of tannin shall agree within 0.6 percent. For solid extracts of 60 percent tannin content, the results shall agree within 1.8 percent. The analysis shall be repeated if necessary until such agreement is reached, and it must be clearly stated on the report that the result is the mean of such determinations. Where analyses are carried out by different chemists on the same sample of extract or tanning material, their results should not differ by more than 3.0 percent of the total tannin content. When reporting analyses, results shall be stated to one place of decimals only.

#### 33. *Conclusion.*

All analyses shall be performed in strict accordance with the foregoing instructions and the report must state that "the analysis has been made by the Official International Method of Tannin Analysis." The lot number of the hide powder used in making the analysis must also be stated on the report.

## APPENDIX

Approximate quantities of materials to be taken for analysis in grams per liter.

## Solid Tanning Materials (Woods, Barks, fruits, leaves, etc.):

Canaigre	15-18	Myrobalans (pulp only)	8-10
Chestnut Wood (fresh)	50-55	Myrobalans (whole nuts)	12-14
Chestnut Wood (dry)	38-42	Valonia (whole cups)	14-15
Quebracho Wood and Tizerah	19-21	Valonia Beard (trynacks)	9-10
Hemlock Bark	32-36	Divi-divi, algarobilla, teri and gonakie	10-12
Mimosa Bark	10-14	Sumach	15-16
Oak Bark	35-45	Spent tans	50-80
Mangrove Bark	10-12		
Pine Bark	30-35		

## Solid Extracts:

Chestnut (60 percent)	6-7	Sumach	6-7
Mangrove	6	Cutch	10
Quebracho (natural)	6	Gambier, cube	12-14
Quebracho (soluble)	6	Gambier, block	14-16
Mimosa Bark	6-7		

## Liquid Extracts:

Chestnut (30 percent)	13	Myrobalans (25 percent)	16
Quebracho (Nat) and (Sol.)	12	Hemlock	11-13
Mimosa Bark	11-13	Pine Bark	13
Oak Wood	16	Sulphite Cellulose (wood-pulp)	16-18
Sumach	16	Synthetic Tannins	13

## Errors in Official Tannin Determination.

It will be apparent from the discussion of the equilibria of protein systems in Chapter 5 that the above described methods involve two false assumptions: one, that the hide powder combines only with tannin; the other that the solution absorbed by the collagen jelly has the same concentration as that in the surrounding solution. It may be mentioned that the former assumption introduces errors vastly greater than the latter. As long ago as 1903, Procter and Blockey<sup>8</sup> showed that hide powder removes from solution considerable amounts of such

TABLE XXXII

RESULTS OF TREATMENT OF PURE GALLIC ACID SOLUTIONS BY A. L. C. A. METHOD.  
(Using 47 grams of wet hide powder (73 percent water) to 200 cc. of solution.)

Gallic Acid Grams Per Liter	Nontannin Percent	Tannin Percent
8.88	54.0	46.0
4.44	47.1	52.9
2.22	43.8	56.2
1.11	40.4	59.6

TABLE XXXIII

EFFECT OF ALTERING PROPORTION OF HIDE POWDER UPON AMOUNT OF GALLIC ACID REMOVED FROM A 0.888-PERCENT SOLUTION.

(Using principle of A. L. C. A. method.)

Wet Hide Powder (73 percent water) Grams Per 200 cc.	Nontannin Percent	Tannin Percent
5	91.8	8.2
10	86.0	14.0
25	69.6	30.4
50	52.1	47.9
75	43.7	56.3

nontannins as gallic acid, quinol, and catechol. Wilson and Kern<sup>15</sup> showed this even more strikingly by subjecting pure solutions of gallic acid to the A.L.C.A. method of tannin analysis. By varying the concentration or the proportion of hide powder, practically any results desired could be obtained. Tables XXXII and XXXIII show that the A.L.C.A. value for tannin decreases with increasing concentration of the solution and increases with the proportion of hide powder. Using a solution of 1 gram of gallic acid per liter, the method indicates a tannin content for the sample of about 60 per cent, even though it contains none at all.

### Original Wilson-Kern Method of Determining Tannin.

With the object of avoiding the errors of the A.L.C.A. method, Wilson and Kern<sup>16</sup> set out to devise a method that would determine exactly what is called for in their practical definition of tannin, namely, that portion of the soluble matter of vegetable tanning materials which will precipitate gelatin from solution and which will form compounds with hide fiber which are resistant to washing. The principle of their method is to shake a convenient amount of the tannin solution with a known quantity of purified hide powder until all tannin has been removed from solution, as determined by the gelatin-salt test. The tanned powder is then washed free from soluble matter including the nontannin removed from solution by the hide powder, which is responsible for the large errors in the A.L.C.A. method. It is then carefully dried and analyzed for tannin as in the regular procedure for vegetable-tanned leathers, and from this figure the per cent of tannin in the original material may readily be calculated.

In order to show the workability of this method, Wilson and Kern selected 8 typical tanning materials showing great differences in prop-

erties, especially in so-called astringency. The solid quebracho extract and the four liquid extracts of oak bark, larch bark, chestnut wood, and osage orange were typical samples of the best of these materials on the American market. The gambier was the ordinary pasty product from the East Indies; the sumac, consisting of ground leaves and small twigs, was from Palermo; and the hemlock bark came from the forests of Wisconsin. The extracts were simply dissolved in hot water, cooled slowly, and made up to the mark. The bark and sumac were finely ground and leached by percolation, only the extracted portions being used after making up to a definite volume. In each test, 12 grams of hide powder (of known hide substance content) were put into a wide-mouthed, rubber-stoppered, half-pint bottle, the tanning material dissolved in 200 cubic centimeters of solution was added, and the whole was shaken in a rotating box for 6 hours.

The amount of material that could be used was limited by the amount of tannin that the hide powder was capable of taking up in 6 hours. On the other hand it was desirable not to use too little, since the less the amount of tannin fixed per unit of hide substance, the less the accuracy of the method, since the tannin was determined by difference. Whenever the liquor, after the 6-hour shaking, gave a turbidity or precipitate with the gelatin-salt reagent, the test was repeated with less material.

The tanned powder was washed by shaking with 200 cubic centimeters of water for 30 minutes, squeezing through linen, and repeating the washing operation until the wash water showed no color and gave no test with ferric chloride solution. Nontannins like gallic acid give a dark coloration upon the addition of ferric chloride solution. Except for the osage orange and chestnut wood extracts, which are unusual in several respects, not more than 12 washings were required to free the powders from nontannin, which shows that the line of demarcation between tannin and nontannin is fairly sharp for the commoner materials. The wash water continued to extract coloring matter from the powders tanned with osage orange until after the fiftieth washing, while as many as 25 washings were required to free the powders tanned with chestnut wood from soluble matter producing a dark color with ferric chloride. All wash water was tested with the gelatin-salt reagent, but in every case the test was negative.

The washed powders were dried at room temperature for 24 hours or longer and then analyzed for water, ash, fat and hide substance. The per cent of hide substance was taken as the per cent of nitrogen multiplied by 5.62. The difference between 100 and the sum of the



TABLE XXXIV

Percentage Analysis of  
Tanned Hide Powder

Material	Material Grams Per Liter	Water	Ash	Fat	Hide Substance (N x 5.62)	Tannin (by difference)	Per 100 g. of Hide Substance		Tannin in Material Percent
							Found Grams	Used Grams	
Quebracho .....	18.8	11.56	0.14	0.35	74.92	13.03	17.39	36.8	47.26
Quebracho .....	18.8	11.42	0.08	0.35	75.05	13.10	17.46	36.8	47.45
Quebracho .....	11.5	13.81	0.03	0.30	77.53	8.33	10.74	22.6	47.52
Hemlock Bark .....	150.0	9.94	0.12	0.24	76.54	13.16	17.19	287.9	5.97
Hemlock Bark .....	100.0	10.73	0.13	0.28	79.39	9.47	11.93	191.9	6.22
Hemlock Bark .....	75.0	12.76	0.05	0.28	79.53	7.38	9.28	147.1	6.31
Oak Bark .....	67.5	12.54	0.07	0.12	74.76	12.51	16.73	131.6	12.71
Oak Bark .....	45.0	11.19	0.09	0.24	79.36	9.12	11.49	87.6	13.12
Oak Bark .....	25.0	13.53	0.05	0.34	81.00	5.08	6.27	48.9	12.82
Larch Bark .....	67.5	12.59	0.09	0.13	75.61	11.58	15.32	131.6	11.64
Larch Bark .....	45.0	13.65	0.09	0.30	77.90	8.06	10.35	87.7	11.80
Larch Bark .....	25.0	16.52	0.08	0.25	78.65	4.50	5.72	48.9	11.70
Chestnut Wood .....	67.5	12.43	0.11	0.05	75.76	11.65	15.38	131.6	11.69
Chestnut Wood .....	45.0	12.82	0.13	0.19	78.54	8.32	10.59	87.7	12.08
Chestnut Wood .....	37.5	12.05	0.10	0.21	80.74	6.90	8.55	71.7	11.92
Sumac .....	93.8	11.39	0.16	0.36	74.92	13.17	17.58	179.3	9.80
Sumac .....	62.5	12.26	0.23	0.31	78.38	8.82	11.25	119.5	9.41
Sumac .....	37.5	11.75	0.12	0.37	81.97	5.79	7.06	73.5	9.61
Osage Orange .....	48.8	12.82	0.13	0.17	77.35	9.53	12.32	95.0	12.97
Osage Orange .....	32.5	12.83	0.09	0.25	80.09	6.74	8.42	63.3	13.30
Osage Orange .....	26.3	12.43	0.12	0.25	81.43	5.77	7.09	51.2	13.85
Gambier .....	50.0	12.08	0.18	0.19	81.44	6.11	7.50	97.4	7.70
Gambier .....	49.5	11.77	0.26	0.28	81.70	5.99	7.33	94.7	7.74
Gambier .....	29.0	13.06	0.14	0.35	82.74	3.71	4.48	56.5	7.93

percentages of water, ash, fat and hide substance was taken as the per cent of tannin in the tanned powder. The parts of tannin per 100 parts of hide substance divided by the parts of tanning material used per part of hide substance gave the per cent of tannin in the original material. The results for the 8 materials examined are given in triplicate in Table XXXIV.

### Comparison of A.L.C.A. and Wilson-Kern Methods.

The A.L.C.A. and Wilson-Kern methods give very different results. A careful comparison is therefore desirable, especially since it will assist in giving a better understanding of the vegetable tanning process and of what is ordinarily called tannin. It should be remembered that the great majority of tannin values quoted in the literature were obtained either by the A.L.C.A. method or by some method based upon similar principles. The Wilson-Kern method is still too new to have found general acceptance.

TABLE XXXV  
PERCENTAGE ANALYSIS OF MATERIAL

Material	Water	A. L. C. A. Method			Wilson-Kern Method Tannin	Percentage Error in A.L.C.A. Method
		Insoluble Matter	Nontannin	Soluble Matter Tannin		
Quebracho .....	17.87	7.16	6.96	68.01	47.41	43
Hemlock Bark ...	8.90	74.33	6.71	10.06	6.17	63
Oak Bark .....	52.66	3.68	19.46	24.20	12.88	88
Larch Bark .....	51.08	5.88	20.90	22.14	11.71	.89
Chestnut Wood ..	58.90	1.50	13.80	25.80	11.90	117
Sumac .....	9.25	47.20	17.99	25.56	9.61	166
Osage Orange ...	46.05	3.45	10.63	39.87	13.37	198
Gambier .....	51.12	5.36	18.57	24.95	7.79	220

For the sake of comparison, Wilson and Kern analyzed the 8 materials they studied by both methods and the results are given in Table XXXV. The percentage error in the A.L.C.A. method is calculated on the assumption that the results of the Wilson-Kern method are correct. Although the enormous errors in the A.L.C.A. method are nothing short of sensational, they are probably not at all exaggerated. But the extent of these errors is less surprising in view of the large proportion of such nontannins as gallic acid that appear as tannin by the A.L.C.A. method, as shown in Tables XXXII and XXXIII.

The need for arbitrary limits in the A.L.C.A. method was clearly shown by the gallic acid experiments, but was more strongly emphasized by similar experiments upon actual tan liquors. The effect of

TABLE XXXVI

EFFECT OF VARIATION IN AMOUNT OF HIDE POWDER USED UPON PERCENT OF TANNIN OBTAINED BY A. L. C. A. METHOD.

Material	Grams Per Liter	Wet Hide Powder (73 percent water) Used to Detannize 200 cc. Tan Liquor Grams	Apparent Percent of Tannin by A. L. C. A. Method	Percentage Error Due to A. L. C. A. Method
Quebracho .....	3	93.3	68.18	44
		46.7	67.56	43
		26.7	66.61	40
		13.3	64.36	36
		6.7	57.56	21
Hemlock Bark ...	20	93.3	10.98	78
		46.7	10.60	72
		26.7	9.76	58
		13.3	9.35	52
		6.7	7.98	29
Oak Bark .....	4.11	93.3	25.02	94
		46.7	24.59	91
		23.3	24.01	86
		11.7	22.09	72
		5.9	18.77	46
Larch Bark .....	4.37	93.3	28.10	140
		46.7	24.52	109
		23.3	21.97	88
		11.7	19.10	63
		5.9	16.24	39
Chestnut Wood .....	15	93.3	26.87	126
		46.7	25.80	117
		26.7	24.59	107
		23.3	23.52	98
		16.0	22.49	89
Sumac .....	4	93.3	24.98	160
		46.7	25.05	161
		23.3	24.47	155
		11.7	23.45	144
		5.9	21.45	123
Osage Orange .....	8	93.3	40.48	203
		46.7	39.47	195
		26.7	38.21	186
		13.3	36.27	171
		9.3	35.67	167
Gambier .....	4.58	93.3	29.04	273
		46.7	25.60	229
		23.3	22.56	190
		11.7	17.22	121
		5.9	13.38	72

altering the proportion of hide powder with solutions of the 8 tanning materials is shown in Table XXXVI and in Figs. 88, 89, and 90. In Figs. 88 and 89 the short, vertical lines are placed at points on the curves corresponding to the smallest amount of hide powder that would completely detannize the solutions under the conditions of the A.L.C.A. method, as determined by the gelatin-salt test. The crosses indicate the points corresponding to the quantity of hide powder called for in the A.L.C.A. method. The zero points represent the percentages

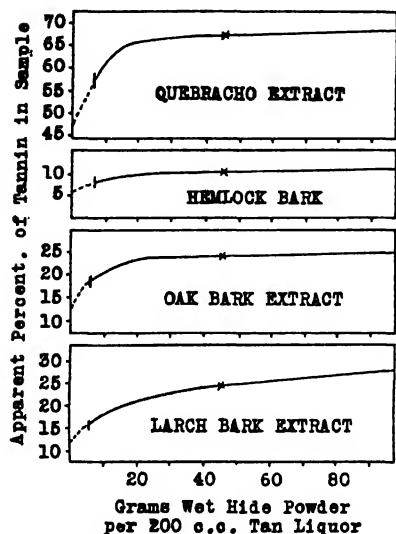


FIG. 88.—Effect of variation in amount of hide powder used upon the determination of tannin by A. L. C. A. method.

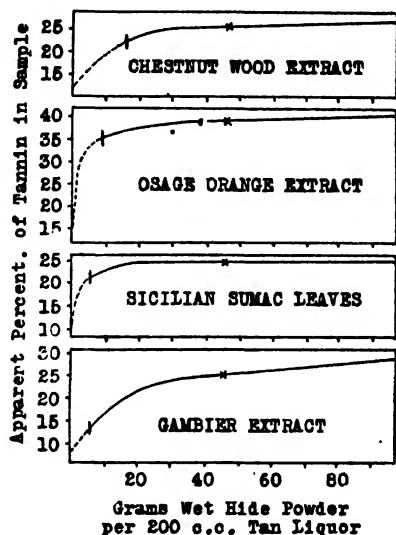


FIG. 89.—Effect of variation in amount of hide powder used upon the determination of tannin by A. L. C. A. method.

of tannin found by the Wilson-Kern method and the broken portions of the curves are extrapolated.

No scientific reason has ever been given for the selection of the particular amount of hide powder called for in the A.L.C.A. method. So far as the principle of the method is concerned, any of the values given in Table XXXVI might be accepted as correct, since the solutions were completely detannized in every case. This should be borne in mind when employing figures for tannin appearing in the literature, which have been found by this or similar methods.

As would be expected, the greatest errors in the A.L.C.A. method are obtained with those materials containing the greatest proportion of nontannin to tannin. Quebracho, having least nontannin, gives the

smallest error. However, if the quebracho is mixed with gallic acid to make the proportion of nontannin to tannin about the same as in the case of the gambier, it gives errors nearly as great as in the case of the gambier, which is shown in Table XXXVII.

Comparison of the two methods has brought out at least one fact of practical significance: Those materials which give the least errors by the A.L.C.A. method are most astringent, while those giving greatest errors are least astringent. The order of the materials in Table XXXV might almost be taken as the order of decreasing stringency, although

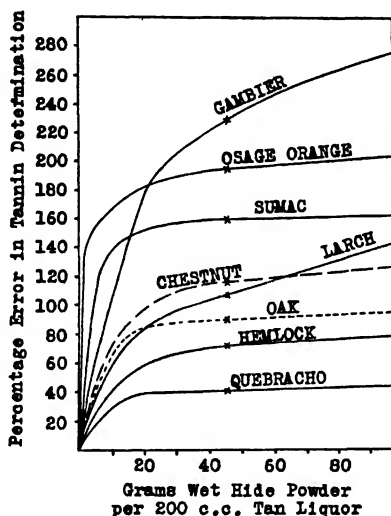


FIG. 90—Effect of variation in amount of hide power used upon the error involved in determining tannin by the A. L. C. A. method.

an exact parallelism cannot be claimed. Quebracho and hemlock bark are generally conceded to be the most astringent and sumac and gambier the least astringent of these materials. This suggested a relation between astringency and the ratio of nontannin to tannin. Astringency appears to be a function of the rate of combination of tannin and protein. In the experiments listed in Table XXXIV the hide powder fixed more than twice as much tannin from the quebracho liquors in 3 hours as from the gambier liquors in 6 hours. But, when enough gallic acid was added to the stronger quebracho liquors to give them the same ratio of nontannin to tannin as in the gambier, the hide powder did not remove anywhere nearly all the tannin in 6 hours. Upon addition of the gelatin-salt reagent to the liquors after the 6-hour shaking, huge precipitates were formed, suggesting a great

TABLE XXXVII

MIXTURE OF QUEBRACHO EXTRACT AND GALLIC ACID.

Wet Hide Powder (73 per- cent water) Used to Detannize 200 cc. Tan Liquor Grams	Percentage Analysis of Mixture of 5 Parts of Quebracho Extract to 9 Parts of Dry Gallic Acid					Percentage Error in A. L. C. A. Method		
	A. L. C. A. Method				Wilson- Kern	Alone (from Fig. 90)	In Pres- ence of Gallic Acid	Gambier Alone
	Water	Insoluble Matter	Soluble Nontannin	Matter Tannin	Method Tannin			
93.3.....	5.80	3.96	33.87	56.37	16.93	44	233	273
46.7.....	5.80	3.96	37.14	53.10	16.93	43	214	229
23.3.....	5.80	3.96	44.07	46.17	16.93	39	173	190
11.7.....	5.80	3.96	53.39	36.85	16.93	33	118	121
5.9.....	5.80	3.96	63.34	26.90	16.93	18	59	72

reduction in astringency. That the effect was only one of slowing up the tanning action was proved by the fact that the hide powder was able to detannize the solution completely in 24 hours. This also explains the mild action of tan liquors which have been used a great many times and have consequently accumulated a large amount of nontannin.

The polemics following the publication of the Wilson-Kern method served to stimulate investigations of the properties of tanning materials. At the 17th annual meeting of the American Leather Chemists Association a formal discussion<sup>13</sup> of the Wilson-Kern method was staged, and the chief aim of the opposition was apparently to show that the low results obtained were due to losses of tannin in the manipulation. It was contended that a certain proportion of the tannin of a liquor will form a stable compound with hide only after long contact, and, further, that even tannin which has already combined with the hide powder will be removed to an appreciable extent during the washing required by the Wilson-Kern method, but no substantial evidence was offered in support of these contentions.

### Effect of Washing.

Certain differences in behavior of the several different tanning materials have caused a widespread belief that some tannins form more stable compounds with skin than others; for example, the tannin from gambier is supposed to form a compound with skin less stable than that from hemlock bark. It has also been supposed that mixtures of tanning materials behave differently in this respect from the individual materials.

Wilson and Kern<sup>17</sup> made a careful study of the possible losses of tannin during the washing operation involved in their method and came to the conclusion that any such loss was too small to have any effect upon the determination. Table XXXVIII shows that practically the same results are obtained for a great variety of tanning materials, whether the tanned hide powders were washed 15, 25, or 50 times. Theoretically, tanning may be reversible, but the rate of hydrolysis is so small as to have no bearing on the Wilson-Kern method, which holds equally well for both mild and astringent tanning materials.

TABLE XXXVIII

EFFECT OF EXCESSIVE WASHING OF THE TANNED HIDE POWDER UPON THE PER CENT OF TANNIN FOUND BY THE WILSON-KERN METHOD.

Extract	Extract Grams in 200 cc. Solution	Hide Substance in Powder Used to Detannize 200 cc. Solution Grams	Percent Tannin in Extract. Value Obtained from Analysis of Tanned Powder Washed		
			15 Times	25 Times	50 Times
Quebracho .....	3.80	10.44	46.84	47.25	46.90
Gambier .....	10.00	10.44	7.87	7.89	7.67
Gambier-quebracho mixture*....	6.90	10.44	20.67	20.34	20.43
Chestnut wood .....	13.60	10.32	...†	13.99	13.93
Hemlock bark .....	13.00	10.32	23.47	23.38	23.50
Chestnut wood-hemlock bark mixture † .....	13.30	10.32	...†	18.73	19.05
Oak bark .....	13.60	10.40	15.52	15.36	15.35
Larch bark .....	13.60	10.32	...†	11.29	11.28
Sumac .....	13.00	10.39	16.36	16.29	16.39
Wattle bark .....	8.00	10.32	24.66	24.16	24.73

\* Mixture of 19 parts solid quebracho extract to 50 of gambier extract.

† Mixture of 68 parts of chestnut wood extract to 65 of hemlock bark extract.

‡ Calculation not made because 15th wash water gave test for nontannin with ferric chloride.

### Conversion of Nontannin into Tannin.

In criticizing the Wilson-Kern method, Schultz<sup>13</sup> said, "We have taken the nontannins and washings and reconcentrated them under a high vacuum to the original volume of 200 cc. and have tanned hide powder with it, and, by the calculations employed, we have found a definite percentage of tannin." He mentioned also that the concentrated liquor gave a positive test for tannin with the gelatin-salt reagent. It might look at first sight as though the detannized liquor and wash waters, before concentrating, really had contained tannin and Schultz evidently so regarded it. Wilson and Kern confirmed Schultz's experimental finding while analyzing a sample of gambier

extract by their new method. The detannized liquor and 15 wash waters, all of which gave no test with the gelatin-salt reagent, were concentrated to 200 cubic centimeters, whereupon they were found to give a bulky precipitate with the reagent. But, when diluted back to 3200 cubic centimeters, they still gave a bulky precipitate with the gelatin-salt reagent, showing that a most important chemical change had taken place during the concentrating.

Another sample of gambier was analyzed by the new method and found to contain 7.94 per cent of tannin. The detannized liquor and 17 wash waters, 3600 cubic centimeters in all, were evaporated to 250 cubic centimeters, analyzed by the new method, and found to contain 5.56 parts of tannin per 100 of original extract, giving the extract a total of 13.50 per cent tannin. The detailed results are given in Table XXXIX.

TABLE XXXIX

## GAMBIER EXTRACT.

Two hundred cubic centimeters of solution containing 9.00 grams of extract were detannized with 12 grams of air-dry hide powder, containing 10.40 grams of hide substance, and then the tanned powder was washed 17 times with a total of 3400 cubic centimeters of water. The residual liquor and wash waters were evaporated to 250 cubic centimeters and used to tan 12 grams of fresh hide powder, which was afterwards washed as usual.

Analysis of Air-dry, Tanned Powder	High Powder Tanned in Original Solution	Concentrated Wash Waters
Water .....	17.31	16.24
Ash .....	0.16	0.14
Fat (chloroform extract) .....	0.39	0.42
Hide substance (N x 5.62) .....	76.86	79.38
Tannin (by difference) .....	5.28	3.82
Per 100 grams hide substance:		
Tannin found, grams .....	6.87	4.81
Material used, grams .....	86.54	86.54
Percent tannin in extract .....	7.94	5.56

Total tannin, either originally present or formed during the concentrating of the wash waters, 13.50 percent.

In order to show that this increased amount of tannin would have combined with the hide powder had it been present in the original solution, Wilson and Kern made up a new solution of this extract, concentrated and diluted back several times, and then analyzed it by the new method, finding 12.69 per cent tannin. If the concentrating had been continued a little longer, the figure 13.50 would probably have been reached or passed. The results are shown in Table XL.



TABLE XL

## GAMBIER EXTRACT.

(Same as noted in Table XXXIX.)

Dissolved 60.00 grams of extract in 1 liter of water. Concentrated to 250 cubic centimeters and diluted back to 1 liter. Repeated 3 times, the fourth time diluting to 2 liters. Two hundred cubic centimeters of diluted solution, containing 6.00 grams of original extract, were detannized with 12 grams of air-dry hide powder containing 10.37 grams of hide substance, which was afterwards washed as usual.

## ANALYSIS OF AIR-DRY TANNED POWDER.

Water .....	18.23
Ash .....	0.18
Fat (chloroform extract) .....	0.42
Hide substance (N x 5.62) .....	75.62
Tannin (by difference) .....	5.55
Per 100 grams hide substance:	
Tannin found, grams ..	7.34
Material used, grams.....	57.86
Percent tannin in extract .....	12.69

In spite of the great change in the tan liquor produced by concentrating, it is not shown to any appreciable extent in the analyses by the A.L.C.A. method shown in Table XLI. Concentrating the tan liquor and diluting back caused a rise in per cent of tannin by the new method from 7.94 to 12.69, but the rise in the A.L.C.A. method is only from 26.14 to 26.40, which difference is so small as even to be attributable to experimental error. The reason for this small difference is probably that the nontannins which are convertible into tannin all combine with the hide powder initially, even though they are easily removed later by washing.

TABLE XLI

## GAMBIER EXTRACT.

(Same as noted in Table XXXIX.)

Both the original liquor noted in Table XXXIX and the specially treated liquor noted in Table XL were appropriately diluted and analyzed by the A. I. C. A. method.

	Percent of Original Extract	
	Original Liquor	Treated Liquor
Insoluble matter .....	7.66	8.62
Nontannin .....	18.33.	17.57
Tannin .....	26.14	26.40

Just what chemical actions are involved in the conversion of non-tannin to tannin must remain a matter for speculation until more data are available; oxidation, condensation, and polymerization may all be involved. It is conceivable that gallic acid might be converted

into digallic acid under suitable conditions, and it seems extremely likely that a polymerized form of digallic acid would have tanning properties. A solution of pure gallic acid gives no test for tannin, but Wilson and Kern found that after boiling for some time it gives a bulky precipitate with the gelatin-salt reagent, and will then apparently tan skin. A detannized solution, which gives no test for tannin, can be made to give a strong test merely by passing oxygen gas through it. Long exposure to air has a similar action. It is evident that the Wilson-Kern method furnishes a valuable means of studying the conversion of nontannin into tannin, and might conceivably be applied to a study of the formation of tannin in nature and to the aging of barks.

### Effect of Aging.

The conversion of nontannin into tannin is apparently responsible for two factors of great importance to tanners of heavy leathers, namely, the time factor in tanning and the aging of leather. In the A.L.C.A. discussion referred to, Alsop<sup>13</sup> remarked that sole leather tanned slowly not only contains more tannin, but actually consumes less tanning material than the rapid tannages. In a private communication to the author, H. R. Procter has called attention to the fact that leather stored for a long time, or aged, before washing contains more tannin than if it had been washed immediately after tanning. A number of critics have said that the Wilson-Kern method is weak because it does not include as tannin all of the material that can be made to combine with hide substance by aging. This argument, however, is weak because the Wilson-Kern method offers a very satisfactory means of studying the aging properties of different tanning materials. An application of the method to such a study is shown in Table XLII for ten commercial extracts or mixtures thereof.

It is interesting to note that in no case does aging for an entire year raise the tannin value to a point as high as that given by the A.L.C.A. method. Aging the gambier-tanned powder for a year raised the tannin content to about the same value as is produced by merely concentrating the liquor before tanning, as indicated in Tables XXXIX and XL. The change taking place upon aging is probably of the same nature as that described above as the conversion of nontannin into tannin.

In the manufacture of vegetable-tanned upper leather, the effect of aging is probably not very marked. In actual practice, Wilson and Kern found barely 50 per cent as much tannin in the leather coming

TABLE XLII

EFFECT OF AGING UPON PER CENT OF COMBINED TANNIN IN LEATHER.

Three 12-gram portions of hide powder were used to detannize 200 cubic centimeters each of the solutions of tanning materials noted in Table XXXVIII. One portion in each case was washed 25 times immediately after tanning and the other two were allowed to dry without washing. Of these one was kept exactly 30 days and then washed 25 times; the other was kept just 1 year and then washed 25 times.

Extract	Tannin as Per Cent of Original Extract			
	By Wilson-Kern		Method	
	In	In	In	By
	Leather Washed Immediately After Tanning	Leather Kept 30 Days Before Washing	Leather Kept 1 Year Before Washing	
Quebracho .....	47.25	53.00	54.59	60.87
Gambier .....	7.89	10.49	13.13	25.61
Gambier-quebracho mixture .....	20.34	23.92	25.34	33.22
Chestnut wood .....	13.99	18.02	18.36	25.70
Hemlock bark .....	23.38	24.87	25.46	26.68
Chestnut wood-hemlock bark mixture .....	18.73	20.45	21.25	25.64
Oak bark .....	15.36	17.23	20.08	26.19
Larch bark .....	11.29	13.22	18.73	22.96
Sumac .....	16.29	17.94	17.96	25.51
Wattle bark .....	24.16	25.89	26.61	33.55

from a certain upper leather yard during a 3-year period as was put into it, according to the analyses by the A.L.C.A. method of the extracts used. About half of the tannin seemed to be mysteriously disappearing until they applied their new method to the control of the yard and found that the amounts of tannin used and those found in the finished leather then checked easily within the limits of experimental error.

In the manufacture of sole leather, one would expect the effects of aging to be much more pronounced. It seems reasonable to suppose that the Wilson-Kern method could be applied to a sole leather yard by keeping the tanned powders in the dried state for a sufficient length of time before washing to correspond to the conditions under which the sole leather was kept. That the A.L.C.A. method is no more reliable for heavy leather work than for upper leather is indicated by the following figures which have been made available to the author: Of 100 lbs. of tannin, as determined by the A.L.C.A. method, that enter the leach house, only 39 lbs. appear as combined tannin in the finished leather. Losses in the spent tanning material, waste liquors, and water soluble matter from the leather were determined only by the A.L.C.A. method, but even with all this taken into consideration,

there remains a large loss that can be accounted for only on the assumption that the A.L.C.A. method gives results much too high.

### Effect of pH Value.

Thompson, Seshachalam, and Hassan<sup>12</sup> made a preliminary study of the effect of adding acetic and hydrochloric acids to extracts of quebracho, mimosa, mangrove, gambier, myrobalans, chestnut wood, and oak wood and found that the addition of small amounts of acid affected practically all of the determinations made. Fig. 91, taken

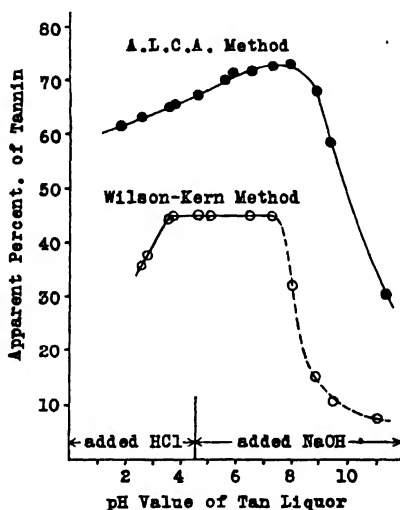


FIG. 91.—Effect of pH value upon the determination of tannin in a sample of quebracho extract.

from a paper by Wilson and Kern,<sup>19</sup> shows how the determination of tannin, by both the A.L.C.A. and the Wilson-Kern methods, is affected by change of pH value. The latter method gives a practically constant value over the wide range 3.6 to 7.3. Where the falling off in per cent of tannin occurs at pH values higher than 7, indicated by the broken line, the results should not be considered as found by this method because in each case the residual solution gave a test for tannin, by the gelatin-salt test, whereas the method specifies that the determination is to be discarded whenever such a test is obtained. The values obtained were included in the curve in order to show the effect of pH value on the rate of tanning.

Following the later work by Rogers,<sup>9</sup> Knowles,<sup>5</sup> Atkin,<sup>1</sup> and others,

the importance of pH value in making official determinations of tannin has come to be recognized generally.

### New Wilson-Kern Method of Determining Tannin.

In order to meet the demand for a simpler and quicker method,\* Wilson and Kern<sup>18</sup> modified their method without any sacrifice in accuracy over the original method, both procedures giving identical results. The procedure in force at the time of this writing follows:

*Preparation of Hide Powder.* A convenient quantity of Standard hide powder, or its equivalent, is washed free from soluble matter with distilled water. It is then soaked in several changes of ethyl alcohol to remove the water and then in xylene to remove the alcohol. It is then air-dried and stored for use. A year's supply may be prepared at one time.

*Tannin Solution.* The tannin solution to be analyzed is prepared exactly as is the solution of solubles in the A.L.C.A. method or in the Provisional International Method, at least 100 cc. of filtrate being collected for each determination.

*Determination of Tannin.* Two grams of the specially prepared hide powder, of known moisture content, are put into a 6-oz., wide-mouth bottle, 100 cc. of the filtered tannin solution added, and the whole put into a rotating box and shaken for 6 hours. It is advisable to keep the liquor and wash water cool to guard against any tendency towards decomposition of the untanned portion of the hide powder. This matter requires attention only in hot weather.

The mixture of powder and liquor is washed into a Wilson-Kern extractor,\* illustrated in Fig. 92. This extractor consists of three glass parts fitting tightly into one another by means of ground joints. A small piece of 10-oz. S.F. white cotton duck filter cloth † is stretched tightly over the bottom outlet of part B and is firmly secured by winding and tying strong thread around the groove. Parts B and C are then put together and the stopcock is opened. The powder and liquor are washed into B, the liquor being allowed to run through the open cock into a beaker and returned until reasonably clear. The clear liquor is tested for tannin by adding, one drop at a time, a freshly prepared solution of 10 g. gelatin and 100 g. sodium chloride per liter. A precipitate indicates that tannin is present, in which case the determination must be repeated, using a more dilute solution of

\* Corning Glass Works, Corning, New York.

† John Boyle & Co., 112 Duane St., New York.

the tanning material. If tannin is absent, the stopcock is closed, B is half filled with water and then fitted to part A with stopcock closed.

The remaining part of the washing apparatus is a reservoir of distilled water set above the extractor, conveniently on the floor above, and connected to the extractor by means of a rubber tube attached to A. The stopcock in A is opened wide and the rate of flow of water is regulated to about 500 cc. per hr. by means of the stopcock in C, which is connected to the drain. Since the washing is usually complete in about 12 hrs., it is convenient to start it just before leaving the

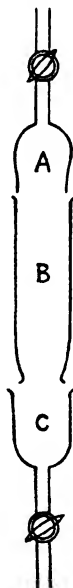


FIG. 92.—Glass washing apparatus for use in Wilson-Kern method of tannin analysis or in determination of water-soluble matter in leather. Total length of extractor when set up is 18 inches.

laboratory in the evening so that it will be complete at the start of the next day. However, washing should not be stopped until the wash water is colorless and does not darken upon the addition of a drop of ferric chloride.

The powder is then washed on to a Büchner funnel and freed from as much water as possible by suction. It is then allowed to dry in the air over night, after which it is completely dried in a vacuum oven for 2 hrs., desiccated and weighed. It is returned to the oven and reweighed as a check against insufficient drying. The increase in weight of the dry powder represents the amount of tannin present in 100 cc. of the original tan liquor.

### Sensitivity of the Gelatin-Salt Test for Tannin.

In testing a solution for the presence of tannin, it is customary to add to it one drop of a solution made by dissolving 10 grams of gelatin and 100 grams of sodium chloride in a liter of water, a precipitate or turbidity indicating the presence of tannin. This reaction has been the subject of numerous investigations for more than a century. Its sensitivity as a means of detecting tannin in solution has recently been studied by Thomas and Frieden.<sup>10</sup> They found that the added gelatin is completely precipitated when the ratio of gelatin to tannin does not exceed 0.5; a great excess of gelatin prevents precipitation.

Thomas and Frieden studied the precipitation of tannin by gelatin at different pH values and concentrations of salt. Using a gelatin solution containing no salt, they obtained a maximum precipitation of gallotannic acid, in pure solution, at a pH value of 4.4; at pH values below 4 or above 5, the solutions became opalescent, but no precipitate formed. The effect of adding sodium chloride was to widen the range of pH value over which a precipitate was obtainable; it apparently had no effect upon the sensitivity of the test between the pH values 4 and 5. Using various commercial tanning extracts, they found that the optimum range for precipitation of tannin by gelatin varied from 3.5 to 4.5, quebracho, wattle, and hemlock precipitating most readily at pH values slightly above 4.0 and gambier, oak, and larch at values slightly below 4.0.

The limits of dilution at which tannin could be detected by means of the gelatin-salt reagent were found to depend upon the proximity of the solution to the optimum pH value for precipitation, which is different for each kind of extract, but apparently always lies between 3.5 and 4.5. At the optimum pH value, gambier, the least sensitive to the test, could be detected at a concentration of 1 part of tannin to 110,000 parts of water. Wattle, the other extreme, could be detected at a dilution of 1 to 200,000. When the commercial extracts were simply diluted with distilled water, no attention being paid to the final pH values, the sensitivity of the tests was greatly decreased. The least sensitive was then hemlock at 1 part in 6,500 and the most sensitive was gambier at 1 part in 30,000. They also found that the age of the gelatin-salt reagent has no effect on the sensitivity of the test, provided bacterial action is prevented by means of toluene.

### Measurement of Astringency.

When a skin is put into a tan liquor, the initial combination of tannin with the surface protein tends to give the outer surfaces an area different from that of the skin as a whole and a tension is produced. When a tan liquor is tasted, this produces on the tongue the sensation of puckering. Materials causing this effect are called astringent in proportion to the vigor of the effect. Astringent tanning materials tan rapidly and so the rate of tanning in a given tan liquor has sometimes been used as a measure of astringency of the liquor. Certain acidic nontannins markedly reduce the astringency of tan liquors. Since a large portion of such material is measured as tannin by the A.L.C.A. method, the discrepancy in tannin found in the A.L.C.A. and Wilson-Kern methods is usually greatest for the least astringent materials.

This has led Crede <sup>4</sup> to suggest defining the astringency of a vegetable tanning material as the ratio of the per cent tannin determined by the Wilson-Kern method to the per cent tannin determined by the A.L.C.A. method. This ratio appears to have been found useful in tanning practice.

### Measurement of Plumping Power.

The yield of vegetable tanned leather depends in some degree upon the plumpness of the skin during tannage. It is, therefore, desirable to know how much the skins will be plumped by a given tan liquor. The degree of plumping is a function of many variables, including the concentrations of hydrogen ion, salts, tannin, and non-tannin. This makes it more satisfactory to measure the plumping effect of any liquor directly upon skin. Many different methods have been proposed, but it will suffice here to describe the two that have been found most useful.

*Wilson-Gallun Method.* The method for measuring the plumping and falling of skin in liquors of varying composition which was devised by Wilson and Gallun <sup>14</sup> is described in detail in Chapters 9 and 10. This method has been used very successfully, in practice, to measure the plumping power of tan liquors. It has the advantage of being able to duplicate tannery conditions. In this method, the degree of plumping of the skin is taken as the ratio of the resistance of the skin to compression under the conditions of the test to its resistance to compression under standard conditions. The great practical value of this method is that the standard condition may be taken as the condition



in which the skins normally enter the tan yard. The test may be so made that the ratio of skin to liquor is the same as obtains in the tan yard. Then comparative measurements with different tan liquors, or with the same liquor treated in different ways, will give an exact indication of the degree of plumping to be expected in practice. A number of measurements made by this method will be described in the chapter on Vegetable Tanning in Volume Two.

*Porter Method.* Porter,<sup>8</sup> working with a committee of the American Leather Chemists Association, found that he could get greater sensitivity in making plumping measurements, by substituting hide powder for actual skin, as used in the Wilson-Gallun method. For each test a glass cylinder is used having an inside diameter of 26 mm. and a total length of 230 mm. Standard hide powder is used which will not pass through a sieve with 40 meshes to the inch. 1.5 grams of this powder are put in the cylinder, covered with 50 cc. of water, and stirred occasionally for several hours. A measuring plunger is made as follows: The lower tube of a 15-cc. pipette is cut off close to the bulb and, in its place, a perforated disk is attached which very nearly fits the bore of the cylinder. The attachment is made so that the pipette can hold a charge of mercury. Enough mercury is poured into the pipette to give the plunger a total weight of 175 grams. The plunger is then introduced into the cylinder so that the perforated disc rests on the hide powder. After exactly 5 minutes the height of the column of hide powder is read off to the nearest tenth of a millimeter. The plunger is then withdrawn, 25 cc. of water siphoned off, 75 cc. of the tan liquor to be tested added, and the mixture is stirred occasionally for 24 hours. Then the plunger is allowed to rest again upon the hide powder, the height of which is measured after 5 minutes. The second reading divided by the first is the measure of the degree of plumping of the hide powder, or of the plumping power of the liquor.

The Porter method is really a modification of the Wilson-Gallun method in which hide powder is substituted for skin. It has the advantage of using a standard hide powder against the more variable skin, but the practical disadvantage of not including the condition of the skin as it enters the tan liquor, which may markedly affect the results

### Ultrafiltration of Tannin Solutions.

R. J. Browne<sup>8</sup> attempted to separate tannin from nontannin by ultrafiltration, assuming a large difference in size between the particles making up these two classes in a tan liquor. He prepared membranes

by immersing disks of filter paper in solutions of 6 per cent pyroxylin in alcohol and ether until the evolution of air bubbles ceased, withdrawing and draining them for a few seconds, moving them horizontally in their own plane to obtain an even surface of collodion, and then immersing them in water. The permeability was found to depend upon the time allowed for drying before immersion in water. Membranes were prepared as permeable as possible without allowing any tannin to pass, as determined by testing the filtrate with the gelatin-salt reagent.

Browne found that such a membrane would pass gallic acid, but not gallotannin. He used the procedure to filter tan liquors, under pressure, and weighed the solid residue from the evaporation of an aliquot of filtrate and calculated it as nontannin. When the method was applied to a number of common tannin extracts, the values for nontannin obtained agreed closely with those obtained by the official method of the Society of Leather Trades' Chemists, which Browne considered as evidence of the validity of the official method.

Thomas and Kelly,<sup>11</sup> however, showed how the ultrafiltration of a vegetable tanning extract varies with the pH value of the solution and with slight changes in manipulation.

Membranes were prepared using the same trade-marked collodion and as closely as possible according to Browne's technic, and used in the ultrafiltration of solutions of hemlock bark extract. The collodion solution was kept in a desiccator, in order to prevent loss of solvent by evaporation.

A stock solution of hemlock bark extract was prepared to contain about 1.6 grams tannin (tannin as defined by the official method of analysis). This stock solution, after adjusting to various pH values by electrometric titration with sodium hydroxide or hydrochloric acid, was diluted so as to contain 0.4 gram tannin per 100 cc. and then ultrafiltered.

The two ultrafilters used were the Columbia type. One was developed in their own laboratory. The other was an improved type made by Hayes and Whitmore, of Urbana, Ill. (Fig. 93.) The pressure was furnished by a tank of compressed nitrogen attached to the ultrafilter through a Hoke pressure reduction valve. Nitrogen gas was used instead of compressed air in order to avoid oxidation of the tannin solutions. The total solids of each solution, before ultrafiltration, were determined by evaporation of a definite volume of sample in the usual way, and the amount of solute passing through the ultrafilter was similarly determined by evaporation of a measured portion

of the ultrafiltrate to dryness. All weights in the table refer to 25 cc. of solution.

In Table XLIII, Series III, at pH = 4.1 and 4.5, are given duplicate

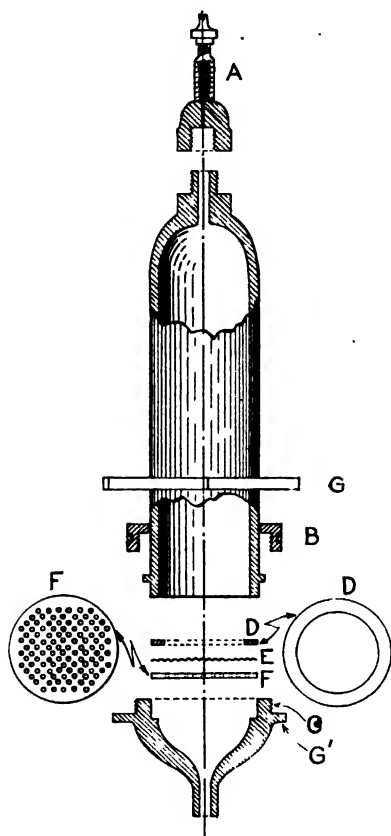


FIG. 93.—Ultrafilter.

- A = automobile tire valve
- B = collar which takes up on funnel at C
- D = rubber gasket
- E = wire gauze on which membrane rests
- F = perforated plate
- G, G' = hexagonal form to hold in vise

filtrations run simultaneously. A deviation of 8 to 10 per cent is seen. This is an example of deviations due to the difference in permeability of two membranes as a result of the uncertain technic of drying to a certain "tackiness."

Series I and II were made with the same hemlock bark solutions,

but the membranes used in Series II were made after a number of others had been prepared from the same 6 per cent collodion solution. This solution, although carefully protected from evaporation, had become concentrated owing to unavoidable slight evaporation of alcohol and ether during the preparation of membranes, thus producing less permeable membranes.

In Series III a different lot of hemlock bark solutions was em-

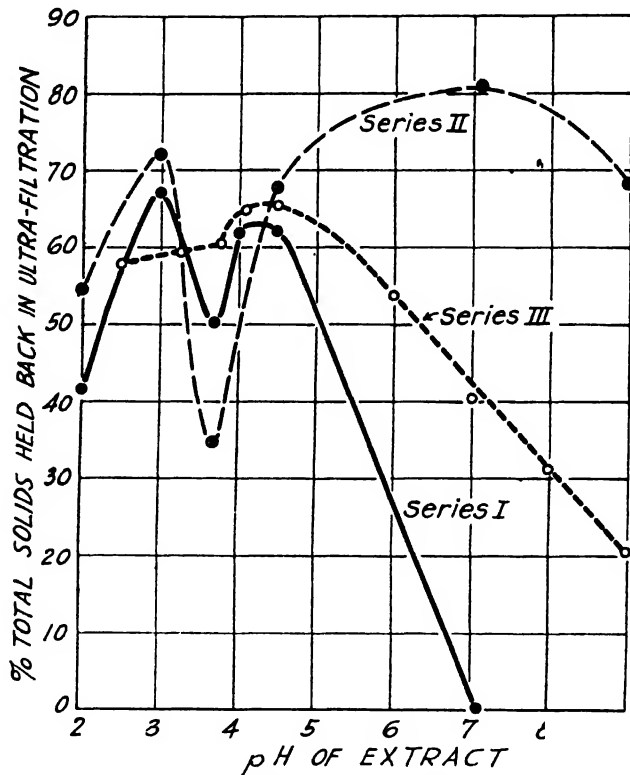


Fig. 94.—Ultrafiltration of hemlock bark.

ployed, but prepared in a manner identical to that of Series I and II. A new 6 per cent collodion solution was also used in Series III; consequently, these membranes were practically identical with those employed in Series I.

Examination of the data and curves in Fig. 94 shows a pronounced difference in ultrafiltration of hemlock bark solutions as a function of the pH thereof. Comparison of the three series reveals large differences in degree of ultrafiltration of identical solutions as a result of

differences in the collodion solutions in which the membranes were prepared and individual variations in the membranes. Furthermore, variations in the gelatin-salt test for tannin (Column 4) give additional indication of the unreliability of this method of separating tannins from nontannins. The depressions in the curves of Series I and II at the "natural" pH of the extract solutions may be due to a coincidence of using less permeable membranes in these cases.

TABLE XLIII

ULTRAFILTRATION OF HEMLOCK BARK TANNING SOLUTIONS OF DIFFERENT pH VALUES

pH (1)	Dry Residue in 25 cc. of Original Soln. Gram. (2)	Ultra- Filtrate <sup>c</sup> Gram (3)	Tannin Test <sup>b</sup> (4)	Solids Held Back in Ultrafilter <sup>c</sup> Gram (5)	Percent of Total Solids Held Back <sup>c</sup> (6)
<i>Series I, Pressure = 80 lbs./sq. in. (234 kg./sq. cm.)</i>					
2.0	0.125	0.073	—	0.052	42
3.0	0.132	0.044	—	0.089	67
3.7 <sup>a</sup>	0.130	0.065	+	0.065	50
4.0	0.131	0.050	++	0.081	62
4.5	0.131	0.050	++	0.081	62
7.1	0.135	0.135	++	0.000	0
9.0	0.140	0.141	++	0.000	0
<i>Series II, Pressure = 200 lbs./sq. in. (586 kg./sq. cm.)</i>					
2.0	0.122	0.055	—	0.067	55
3.0	0.131	0.037	—	0.095	72
3.7 <sup>a</sup>	0.132	0.066	+	0.046	35
4.5	0.133	0.043	—	0.090	68
7.1	0.131	0.025	—	0.106	81
9.0	0.138	0.040	—	0.094	68
<i>Series III, Pressure = 120 lbs./sq. in. (352 kg./sq. cm.)</i>					
2.5	0.130	0.055	+	0.075	58
3.3	0.134	0.055	+	0.079	59
3.8 <sup>a</sup>	0.132	0.052	+	0.080	61
4.1	0.132	0.049	+	0.083	64
4.1	0.132	0.045	—	0.087	
4.5	0.132	0.044	—	0.088	
4.5	0.132	0.048	—	0.084	65
6.0	0.137	0.064	—	0.074	54
7.0	0.138	0.082	+	0.056	41
8.0	0.140	0.097	++	0.044	31
9.0	0.148	0.118	++	0.030	20

<sup>a</sup> "Natural" pH of tannin solution.

<sup>b</sup> The signs in this column refer to the result of a gelatin-salt test upon the ultrafiltrate.

<sup>c</sup> The data in Columns 5 and 6 would be termed "Tannins and Insoluble Solids," and those in Column 3, "Nontannins" by Browne.

The total solids or grams of dry residue in Column 4 are low at pH = 2 and high at pH values alkaline to pH = 7. This is because (a) at pH = 2 hydrolysis on account of the acidity results in the for-

mation of certain volatile organic matter, and (b) in the solutions alkaline to  $\text{pH} = 7$  oxidation of the organic matter increases the weight of solids.

Thomas and Kelly concluded that ultrafiltration of vegetable tanning solutions as a method of separation of tannins from nontannin is of very doubtful analytical value.

### Measurement of Color Value.

Tanners are interested in knowing what color a given tanning extract will impart to leather tanned in it, and so chemists have attempted to measure what is known as the color value of the extract. Formerly it was customary to tan a sheep or calf skiver in a solution of the extract, under fixed conditions, and to note the color. Later a method was developed for measuring the color of the solution by comparison with standard colored glasses. Procter<sup>7</sup> and Blackadder<sup>2</sup> have independently suggested methods based upon the use of the red, yellow, green, and blue regions of the spectrum. The principle involved in Blackadder's method is the measurement of the amount of light passed by a solution of the material under standard conditions. Four separate measurements are made in the following divisions of the spectrum: red wave length 600 to 700, yellow wave length 550 to 600, green wave length 510 to 550, and blue wave length 400 to 510.

Of course, it must be remembered that the same extract can produce a variety of shades of color on skin, dependent upon the condition of the skin and the temperature and concentrations of acid, salt, tannin, and nontannin in the liquor. The effect of pH value was described in Chapter 12.



## Chapter 14.

### Chemistry of the Tannins.

The substances present in barks, woods, and other forms of plant life which are capable of combining with skin protein to form leather are called *tannins*. In aqueous solutions, they have an astringent taste, give dark blue or green colorations with iron salts, and precipitate gelatin, other soluble proteins, and alkaloïds from solution. Attempts to isolate the pure tannins and to study their organic structures have proved exceedingly difficult. It is remarkable that the greatest work on the organic chemistry of the tannins was accomplished by the same man who did most to elucidate the complex structure of the proteins, the late Emil Fischer.

#### Organic Chemistry of the Tannins.

As early as 1852, Strecker<sup>42</sup> had concluded that tannin was a compound of glucose and gallic acid. He was supported by the works of van Tieghem,<sup>43</sup> who found glucose among the hydrolytic products of tannin, and of Pottevin,<sup>40</sup> who effected the hydrolysis with the enzymes of *Aspergillus niger*. But the variation in proportion of glucose found weakened this view, which gave way, for a long time, to that of Schiff,<sup>41</sup> who regarded tannin simply as digallic acid. Although Schiff's formula for tannin was widely accepted, it was finally shown very definitely that digallic acid is not tannin. His formula showed no asymmetric carbon atom in the molecule to account for the optical activity of the natural tannin and it could not account for the high molecular weights observed. By measuring the electrical conductivities, light absorption, and behavior towards arsenic acid, Walden<sup>47</sup> showed that Schiff's digallic acid is very different from natural tannin.

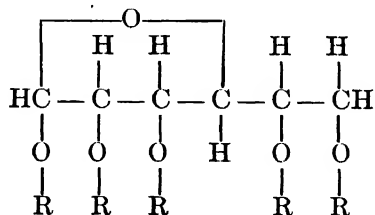
Fischer<sup>8</sup> and his coworkers first set out to determine whether the glucose found by Strecker was really a constituent or only a chance impurity of tannin. They started with the purest technical tannin available. Assuming that the tannin molecule had no carboxyl group, they proceeded to separate it from acid impurities by rendering its



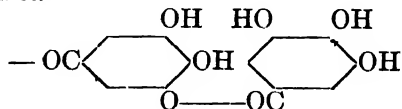
solution slightly alkaline and extracting it with ethyl acetate, a method discovered independently and published previously by Paniker and Stiasny.<sup>36</sup> As they had anticipated, the tannin dissolved in the ethyl acetate, leaving the sodium gallate in the aqueous solution. They accepted this as proof that the tannin possessed no free carboxyl group. Applying this method of purification to several different kinds of commercial tannin, they obtained products that were practically identical.

After hydrolyzing the purified tannin with sulfuric acid, they found between 7 and 8 per cent of glucose. In the purest sample of tannin examined, they found one molecule of glucose combined with ten molecules of gallic acid. No phenolcarboxylic acid other than gallic could be found in tannin, even when the hydrolysis was effected by means of alkali. With excess of alkali and exclusion of air, large yields of alkali salt of gallic acid were obtained in relatively pure condition.

It appeared to Fischer<sup>9</sup> that the surest way to prove his assumptions regarding the structure of tannin was to synthesize it. He started out with the idea that tannin contains no carboxyl and that, consequently, the gallic acid must all be bound as an ester, a condition that would be fulfilled by regarding tannin as an ester-like combination of one molecule of glucose with five molecules of digallic acid, after the manner of pentacetyl glucose. He succeeded in preparing penta-*m*-digalloyl- $\beta$ -glucose, which was proved to be an isomer of the tannin from Chinese nutgalls. The formula for the so-called gallotannic acid may thus be written



where R is the radical



Fischer's success spurred on studies of tannins obtained from a great variety of plant life and it was found that tannins from different sources may differ considerably in composition and in properties.

Although the chemistry of these exceedingly complex substances is just in its infancy, the literature is so voluminous as to require a book of fair size for adequate treatment. It must suffice here to give merely a summary in the form of tables, following the plan of Wilson and Thomas <sup>48</sup> in International Critical Tables.

In tabulating the physical and chemical constants of tanning substances of vegetable origin, one is confronted by many difficulties. The literature is not always clear because of confusion in terminology; *e.g.*, chestnut tannin may mean a raw extract of the wood, bark or leaves, or products obtained therefrom of varying degrees of purification and separation from organic compounds which are not tannins. The term catechin, or catechol, may mean *d*-catechin, *l*-catechin, or *d,l*-catechin. Uncertainty exists also as to the authenticity of the specimen studied in many cases. Perkin and Everest <sup>38</sup> complain of this in connection with the tannins of oak wood and oak bark. In questioning the deductions made concerning the formula of acacia catechin, Nierenstein <sup>35</sup> pointed out that cutch, one of the chief sources of catechin, is obtained from *Acacia catechuoides* and *Acacia sundra* as well as from *Acacia catechin*, despite the general acceptance of the latter as the source. Where exact ultimate formulas are given in the tables below, they are valuable only in indicating the percentages of C, H, and O, except where molecular weight figures are available.

The natural tannins were formerly divided into two classes, the pyrogallol tannins, which gave a blue coloration with ferric salts, and the catechol tannins, which gave a green coloration with ferric salts. This classification has outlived its usefulness and has been supplanted by the more comprehensive systems of Perkin <sup>38</sup> and of Freudenberg.<sup>13</sup>

### Perkin's Classification.

Perkin divides the tannins into three groups: the  $\alpha$  group contains the depsides, or gallotannins; the  $\beta$  group the diphenylmethyloids; or ellagitannins; and the  $\gamma$  group the phlobatannins, or catecholtannins. These are characterized by the following reactions: *FeCl<sub>3</sub>*:  $\alpha$ , blue;  $\gamma$ , green. *Boiling dilute H<sub>2</sub>SO<sub>4</sub>*:  $\alpha$ , gallic acid is formed;  $\beta$ , ellagic acid ppts.;  $\gamma$ , phlobaphenes or "reds" ppt. *Br*:  $\gamma$  give a ppt. *HCl and pine wood*:  $\gamma$  give phloroglucinol reaction, while  $\alpha$  and  $\beta$  do not. *C<sub>6</sub>H<sub>5</sub>N.NCl*:  $\gamma$  give a ppt., indicating the presence of phloroglucinol or resorcinol groups, while  $\alpha$  and  $\beta$  do not. *Fusion with alkali*:  $\alpha$  yield gallic acid and a little pyrogallol;  $\gamma$  yield protocatechuic acid. *Heating in glycerol*:  $\alpha$  form pyrogallol;  $\gamma$  form catechol. *HCHO and HCl*:  $\gamma$

give complete precipitation, the others do not. *Lead acetate* in  $CH_3CO_2H$ :  $\alpha$  are pptd.,  $\gamma$  are not.

### Freudenberg's Classification.

Freudenberg divides the tannins into two main categories, each with three groups, as follows:

**A.** Hydrolyzable tannins in which the benzene nucleus is united to a larger complex through the O atoms. **A1.** Mutual esters of phenol-carboxylic acids or with other hydroxy-acids (Depsides). **A2.** Esters of phenolcarboxylic acids with polyatomic alcohols and sugars. **A3.** Glucosides. In this group gallic acid predominates as the phenolic component. There is also the extraordinary distribution of combined caffeic acid and the presence of a new phenolcarboxylic acid in chebulinic acid. The ellagic acid glucosides also belong here. The most important criterion for the inclusion in this group is the splitting into simple components by hydrolyzing enzymes, especially tannase and emulsin.

**B.** Condensed tannins in which C linkages hold the nuclei together. These are not decomposed into simple components by enzymes. They are generally, not always, precipitated by bromine and under the influence of oxidizing agents or strong acids condense to high molecular weight tannins or "reds." By drastic treatment, preferably by alkalis, the C skeleton is broken up and phloroglucinol, if present, is dissolved out while the remainder of the molecule is transformed mainly into phenolcarboxylic acids. **B1.** Simple ketones such as hydroxybenzophenones and hydroxyphenylstyryl ketones. **B2.** This group is more complicated. The phloroglucinol and benzene nuclei are present in equimolecular proportions. This class embraces the catechols with their corresponding tannins and "reds." This is the most important class of technically used tannins. **B3.** There is practically nothing that can be said about this class of condensed tannins. It is even impossible to state whether they are really jointly condensed systems. In common with the first class of the condensed tannin group, they are precipitated by bromine and are transformed into "reds." On the other hand, they contain no phloroglucinol nucleus. It is possible that the hydroxycinnamic acids are characteristic components of this class; caffeic acid itself is readily transformed into condensation products of the nature of "reds."

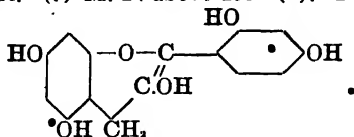
In the following tables, the information is given in the following

order: Name; classification (Perkin's being indicated by Greek letters, Freudenberg's by the above combinations of letters and figures); (1), source; (2) color and form in which it is isolated; (3) formula; (4) solvents; (5) specific rotatory power; (6) color with ferric salts; (7) remarks as to constitution, etc.

TABLE XLIV  
CLASSIFICATION OF THE NATURAL TANNINS

- Beech tannin.**  $\gamma$ . (1) Bark of red beech. (3)  $C_{20}H_{22}O_6$  <sup>(38)</sup>.
- Caffetannic acid.**  $\gamma$ . (1) Coffee berries as Ca and Mg salts; cainia root, *Chiococca brachiata*; *Nux vomica*; St. Ignatius beans; Paraguay tea, *Ilex paraguensis*. (2) Amorphous powder. (4)  $H_2O$ ,  $C_2H_5OH$ . (6) Dark green <sup>(39)</sup>.
- Callutannic acid.**  $\gamma$ . (1) Heather, *Calluna vulgaris*. (2) Amber colored powder. (6) Dark green <sup>(38)</sup>.
- Canaiigre tannin.**  $\gamma$ . B3. (1) Tuberous roots of the sorrel, *Rumex hymenosepalus*. (2) Bright yellow powder. (3) C, 58.10; H, 5.33 <sup>(38)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$ . (6) Green <sup>(13, 38)</sup>.
- Catechol.**  $\gamma$ . B2. (6) Green. See Table XLVI.
- Chebulinic acid, Eutannin.**  $\alpha$ . A2. (1) Myrobalans, fruit of the *Terminalia Chebula*. (2) Rhombic prisms, also colorless needles <sup>(38)</sup>. (3) C, 50.60; H, 3.65. Probably  $C_{24}H_{30}O_{10}$  <sup>(16)</sup>. Air-dry substance contains 16.5%  $H_2O$  of crystn., which is lost at  $100^\circ$  <sup>(4)</sup>. Mol. wt. by titration and by boiling point elevation in acetone, 806 <sup>(16)</sup>. (4) Hot  $H_2O$ ,  $C_2H_5OH$ , acetone, ethyl acetate <sup>(13)</sup>. (5)  $\alpha_D$ ,  $+61.7^\circ$  to  $66.9^\circ$  ( $H_2O$ ) <sup>(4)</sup>.  $\alpha_D^{18}$ ,  $+85^\circ \pm 4^\circ$  (abs.  $C_2H_5OH$ ) <sup>(11)</sup>;  $\alpha_D^{25}$ ,  $-60^\circ$  (acetone, 1%) <sup>(2)</sup>.  $\alpha_D$   $+59^\circ$  to  $67^\circ$  ( $C_2H_5OH$ , 1-2%) <sup>(13)</sup>. (6) Blue-black. (7) Apparently union between di-gallolyl-glucose and the dibasic acid,  $C_{14}H_{14}O_{11}$  with elimination of  $2H_2O$  <sup>(11, 16)</sup>. D. at  $234^\circ$  <sup>(38)</sup>.
- Cherry bark tannin.**  $\gamma$ . (1) Bark of *Prunus cerasus*. (3)  $C_{24}H_{26}O_{10} \cdot 0.5H_2O$ . (6) Green <sup>(38)</sup>.
- Chestnut tannin.**  $\alpha$ . A3. (1) Leaves, bark and wood of Spanish chestnut, *Castanea vesca*. (3) Purified tannin, C, 50.79; H, 3.32. Mol. wt. 400 or multiple as detd. by titration <sup>(25)</sup>. (4)  $H_2O$ . (6) Dark green to blue. (7) Tannin from leaves, wood and bark identical. Raw tannin is mixture containing quercetin, sugar, ellagic and gallic acids. Contains no phloroglucinol. Probably similar to tannin of German native oak <sup>(25)</sup>.
- Chinese tannin.** See Gallotannin.
- Chlorogenic acid.** A1. (1) Monopotassium salt combined with one molecule of caffeine in coffee beans. (2) Cryst. (3)  $C_{16}H_{18}O_6 \cdot 0.5H_2O$ . (4) Hot  $H_2O$ ,  $C_2H_5OH$ , acetone, ethyl acetate. (5)  $\alpha_D$ ,  $-33.1^\circ$  ( $H_2O$ , 1-3%) <sup>(4)</sup>. (6) Green ppt. (7) 3, 4-Dihydroxy-cinnamoyl-quinic acid <sup>(13)</sup>.
- $$\begin{array}{c} \text{HO} \qquad \qquad \text{H} \quad \text{H} \quad \text{O} \\ \qquad \qquad \qquad | \quad | \quad || \\ \text{HO} \text{---} \text{C}_6\text{H}_4 \text{---} \text{C} = \text{C} \text{---} \text{C} \text{---} \text{O} \text{---} \text{C}_6\text{H}_7(\text{OH})_3 \text{---} \text{COOH} \end{array}$$
- Cinchona tannin, quinotannic acid.**  $\gamma$ . B3. (1) Cinchona bark. (2) Light yellow powder. (3)  $C_{14}H_{16}O_6$  (?) <sup>(38)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$  <sup>(13)</sup>. (6) Green ppt. (7) Very hygroscopic.
- Cocatannic acid.**  $\gamma$ . (1) Leaves of *Erythroxylon coca*. (2) Yellow micro. cryst. <sup>(38)</sup>. (3)  $C_{17}H_{22}O_{10} \cdot 2H_2O$  (?). (6) Green <sup>(38)</sup>.
- Colatein.**  $\gamma$ . B2. (1) Cola nuts, *Cola acuminata*. (4) Hot  $H_2O$ ,  $C_2H_5OH$ , acetone. (6) Green. (7) M. P.  $257^\circ$ – $288^\circ$  <sup>(13)</sup>.
- Colatin, Colatannin.**  $\gamma$ . B2. (1) Cola nuts, *Cola acuminata*. (2) Cryst. <sup>(13)</sup>. Light red amorphous powder <sup>(38)</sup>. (3)  $C_{16}H_{20}O_6$  <sup>(38)</sup>. (4)  $C_2H_5OH$ , acetone, ethyl acetate. (5) Inactive. (6) Green. (7) M. P.  $148^\circ$  <sup>(13)</sup>.
- Cortepinitannic acid.**  $\gamma$ . (1) Bark of Scotch fir, *Pinus sylvestris*. (2) Bright red powder. (3)  $C_{32}H_{34}O_{17}$ . (6) Intense green <sup>(38)</sup>.

**Cyanomac lurin.** B2. (1) Wood of *Artocarpus integrifolia*. (2) Cryst. (3)  $C_{18}H_{18}O_6$ . (6) Violet. (7) M. P. above  $290^{\circ}$  <sup>(12)</sup>. Formula: <sup>(27)</sup>



**m-Digallic acid.**  $\alpha$ . A1. (1) Esterified with glucose in Chinese tannin; also synthetic. See Table XLV.

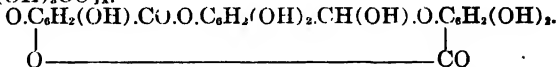
**Ellagic acid.**  $\beta$ . (1) From many tannins containing ellagitannin by boiling with dilute  $H_2SO_4$ . Divi-divi, myrobalans and valonia best sources; also synthetic. See Table XLV.

**Filittannin.**  $\gamma$ . B2. (1) Fern-root, *Aspidium filix-mas*. (2) Red-brown powder <sup>(28)</sup>. (3)  $C_{44}H_{36}NO_{18}$  (?) <sup>(22, 28)</sup>. (4)  $C_2H_5OH$ ,  $H_2O$  <sup>(22)</sup>. (5) Inactive <sup>(12)</sup>. (6) Olive-green. (7) Heated at  $125^{\circ}$ , loses water and becomes insoluble <sup>(12)</sup>.

**Fraxitanic acid.**  $\gamma$ . (1) Leaves of ash tree, *Fraxinus excelsior*. (2) Brownish-yellow deliquescent powder. (3)  $C_{26}H_{18}O_{14}$  (?) <sup>(22, 28)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$  <sup>(22, 28)</sup>. (6) Dark green ppt. (7) Heated at  $100^{\circ}$ , loses water and becomes practically insoluble. Yields quinone upon oxidation by permanganate <sup>(22, 28)</sup>.

**Galitanic acid.**  $\gamma$ . (1) Bark of *Galium verum*. (3)  $C_{14}H_{10}O_{10} \cdot H_2O$ . (6) Green <sup>(28)</sup>.

**Gallotannin, Gallotannic acid, Tannin, Tannic acid.**  $\alpha$ . A2. (1) From galls on leaves and buds of various species of oak, especially *Quercus infectoria* and *Q. lusitania* ("Turkish tannin") due to puncture by insects of the genus *Cynips*. From galls on leaves and buds of a species of sumach, *Rhus semialata* ("Chinese tannin") due to puncture of insect, *Aphis chinensis*. (2) Light yellow-brown powder. (3) Average of several specimens, C, 52.59 to 53.70; H, 3.24 to 3.40 (%).  $C_{44}H_{32}O_{28}$  (F. Fischer). Mol. Wt., 1247-1636 by boiling point elevation in acetone. (4)  $H_2O$ ,  $C_2H_5OH$ , ethyl acetate. (5)  $\alpha_D^{25}$ ,  $+58^{\circ}$  to  $+70^{\circ}$  (different specimens,  $H_2O$ );  $\alpha_D^{20}$ ,  $+18^{\circ}$  (one specimen,  $C_2H_5OH$ ) <sup>(4, 7)</sup>;  $\alpha_D^{22}$ ,  $+129^{\circ}$  (acetone);  $\alpha_D^{22}$ ,  $+176^{\circ}$  (purified specimen,  $C_2H_5OH$ ) <sup>(4, 6)</sup>. (6) Bluish-black. (7) Hydrolysis of purified specimen by dil.  $H_2SO_4$  yields 93.6% gallic acid and 6.8% glucose <sup>(4, 6)</sup>. Undoubtedly a mixture of at least two individuals <sup>(26)</sup>. The tannin, according to E. Fischer, is penta-m-digalloylglucose. Nierenstein objects, asserting that gallotannin is probably a glucoside of polydigalloyl-leucodigallic acid anhydride or of its free acid <sup>(24)</sup>,



**Gallotannin, Chinese Tannin.**  $\alpha$ . A2. (1) See Gallotannin above. (2) Amorphous yellow to light brown powder. (3) Penta-m-digalloylglucose. Mol. wt., 1700 <sup>(12)</sup>. (4) See Gallotannin above. (5)  $\alpha_D^{25}$ ,  $+73^{\circ}$  (purified specimen,  $H_2O$ , 1%) <sup>(4, 7)</sup>;  $\alpha_D$ ,  $+45^{\circ}$  to  $+53^{\circ}$  ( $H_2O$ , 20%), rising rapidly on dilution to  $+135^{\circ}$  to  $+140^{\circ}$  ( $H_2O$ , 1.2%) <sup>(14)</sup>;  $\alpha_D$  in formamide,  $+13^{\circ}$ ; acetone,  $+14^{\circ}$ ;  $C_2H_5OH$ ,  $+18^{\circ}$ ; glacial acetic acid,  $+25^{\circ}$ ; pyridine,  $+40^{\circ}$ . These all showed high and low  $\alpha_D$  fractions in water; were alike in organic solvents. Colloidal forms and impurities markedly affect  $\alpha_D$  in water <sup>(21)</sup>. Two fractions—(a)  $\alpha_D$ ,  $+30^{\circ}$  to  $+40^{\circ}$  ( $H_2O$ );  $+40^{\circ}$  to  $+41^{\circ}$  (pyridine); (b)  $\alpha_D$ ,  $+150^{\circ}$  to  $+158^{\circ}$  ( $H_2O$ );  $+50^{\circ}$  to  $+51^{\circ}$  (pyridine) <sup>(20)</sup>. Purified tannin, after removing part difficultly soluble in water,  $\alpha_D$ ,  $+13.9^{\circ}$  ( $C_2H_5OH$ , 3%);  $+14.9^{\circ}$  ( $C_2H_5OH$ , 10%);  $+13.1^{\circ}$  (acetone, 10%) <sup>(4, 6)</sup>. Potassium salt, containing 10.2% K,  $\alpha_D^{18}$ ,  $+46.3^{\circ}$  ( $H_2O$ , 1%) <sup>(4, 6)</sup>. (6) Bluish-black. (7) Upon hydrolysis with dil.  $H_2SO_4$  there is produced 88.6% gallic acid and 11.4% glucose <sup>(12)</sup>. This tannin is a mixture of deka-, nona-, and octa-galloylglucoses averaging 8 to 9 gallic acid radicals to 1 molecule glucose. The fractions of lower  $\alpha_D$  contain more depside-like gallic acid <sup>(20)</sup>. See also gallotannin above.

**Gallotannin, Turkish Tannin.**  $\alpha$ . A2. (1) See Galotannin above. Aleppo galls. (2) Amorphous yellow to light brown powder. (3) C, 52.5; H, 3.5 (<sup>29</sup>). (4) See Gallotannin above. (5)  $\alpha_D^{25}$ , 2.5° (H<sub>2</sub>O, 7%);  $\alpha_D$ , +5° (H<sub>2</sub>O, 7% and less);  $\alpha_D^{24}$  +23.2° to +24.2° (acetone, 10%) (<sup>4</sup>,<sup>7</sup>). (6) Bluish-black. (7) Hydrolysis of purified specimens with dil. H<sub>2</sub>SO<sub>4</sub>: 81.8 to 84.8% gallic acid; 2.7 to 3.8% ellagic acid; 11.5 to 13.8% glucose; 2.0 to 4.1% tannin residue (<sup>4</sup>,<sup>7</sup>). Hydrolysis and fractionation give a series of fractions of increasing  $\alpha_D$  in alcohol from 15.7° to 43.7°. Concomitantly there is a decrease in ellagic and increase in gallic acid content. The ellagic acid is a part of the tannin molecule. At least 25% of the gallic acid is in depside form, partly directly bound in ester form to the sugar hydroxyl groups (<sup>21</sup>).

**Gallnut Tannin.**  $\alpha$ . A2. (1) Galls on acorn cups of *Quercus robur* and *Q. pedunculata*. (3) C, 52.0; H, 3.3 (<sup>23</sup>). (7) Undoubtedly identical with gallotannin.

**Hamamelitannin.**  $\alpha$ . A2. (1) Bark of *Hamamelis virginica*. (2) Fine white needles (<sup>24</sup>). (3) C, 49.9; H, 4.0; H<sub>2</sub>O of crystn. 17.9%, approximating C<sub>28</sub>H<sub>24</sub>O<sub>14</sub>·6H<sub>2</sub>O (<sup>18</sup>,<sup>28</sup>). (4) Hot H<sub>2</sub>O, C<sub>2</sub>H<sub>5</sub>OH, acetone, ethyl acetate (<sup>23</sup>). (5)  $\alpha_D^{25}$ , +29° (H<sub>2</sub>O, 2.35%);  $\alpha_D^{23}$ , +33° (H<sub>2</sub>O, 1.24%);  $\alpha_D^{20}$ , +35.6° (another specimen, H<sub>2</sub>O, 1.2%) (<sup>19</sup>). (7) Contains no free carboxyl group. Acidity, equal to that of pyrogallol, is due to phenolic hydroxyls (<sup>18</sup>,<sup>24</sup>). Upon hydrolysis with dil. H<sub>2</sub>SO<sub>4</sub>: gallic acid, 70%; sugar, 30%. Upon hydrolysis with tannase: gallic acid, 66%; sugar, 34% (<sup>13</sup>). M. P., 115°–117° (air-dry); 203° (dried at 100°) (<sup>28</sup>).

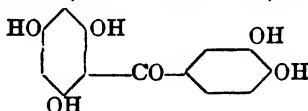
**Hemlock tannin.**  $\gamma$ . (1) Hemlock bark, *Tsuga (Abies) canadensis*. (3) C<sub>28</sub>H<sub>24</sub>O<sub>14</sub> (?). (7) Probably related to quercitannic acid of the oak (<sup>28</sup>,<sup>29</sup>).

**Horsechestnut tannin.**  $\gamma$ . (1) Nearly all parts of the *Aesculus hippocastanum* and in root bark of apple tree. (2) Nearly colorless powder. (3) C<sub>28</sub>H<sub>24</sub>O<sub>14</sub>. (6) Green (<sup>28</sup>).

**Ipecacuanhic acid.**  $\gamma$ . (1) Roots of *Psychotria ipecacuanha*. (2) Reddish-brown hygroscopic substance. (3) C<sub>28</sub>H<sub>24</sub>O<sub>14</sub>. (6) Green (<sup>28</sup>).

**Larch tannin.**  $\gamma$ . (1) Bark of the larch, *Larix europea*. (6) Green (<sup>28</sup>).

**Maclurin.** B1. (1) Wood of "old fustic," *Chlorophora tinctoria*; also synthetic. (2) Yellow crystals (<sup>23</sup>). Colorless needles when pure (<sup>28</sup>). (3) C<sub>18</sub>H<sub>16</sub>O<sub>8</sub>. (4) 14°, 1 part in 190 parts H<sub>2</sub>O (<sup>13</sup>). (6) Green. (7) 2, 4, 6, 3', 4'-Pentahydroxybenzophenone, M. P. 200° (anhydrous form) (<sup>28</sup>).



**Maletto tannin.**  $\gamma$ . B2. Bark of *Eucalyptus occidentalis* and other species of *Eucalyptus*. (2) Brown powder. (3) (C<sub>28</sub>H<sub>24</sub>O<sub>14</sub>)<sub>n</sub> (<sup>28</sup>,<sup>29</sup>). (4) H<sub>2</sub>O, abs. C<sub>2</sub>H<sub>5</sub>OH (from which it is precipitated by ether) (<sup>23</sup>). (7) Similar to quebracho tannin (<sup>13</sup>,<sup>28</sup>).

**Mangrove tannin.**  $\gamma$ . B2. (1) *Rhizophora mangle*, *R. mucronata*, *Ceriops candollana*, *C. roxburghiana*. (2) Amorphous red powder. (3) C<sub>28</sub>H<sub>24</sub>O<sub>14</sub> (<sup>23</sup>). (6) Green. (7) Closely resembles catechutannic acid (<sup>28</sup>).

**Mimosa tannin.**  $\gamma$ . (1) Various species of *Mimosaceae* such as *Acacia arabica* of Egypt and the "wattles" of Australia. (6) Bluish-violet. (7) With the exception of the reaction with ferric salts, gives all the ordinary reactions of the phlobatannins (<sup>28</sup>).

**Oak tannin.** B3 (<sup>26</sup>). (1) Leaves and buds of German oak, *Quercus pedunculata*. (2) Amorphous reddish-yellow powder (<sup>28</sup>). (3) C, 49.9; H, 4.2. (4) H<sub>2</sub>O, C<sub>2</sub>H<sub>5</sub>OH, acetone (<sup>28</sup>,<sup>29</sup>). (4)  $\alpha_{H_2O}^{25}$ , -39° ± 10° (H<sub>2</sub>O); -30° ± 4° (CH<sub>3</sub>OH) (<sup>24</sup>). (7) Tannin from leaves of *Quercus sessiflora* identical (<sup>24</sup>). Molecule contains 18–25% bound ellagic acid; 3–7% bound glucose; and the rest is an amorphous acid, "Quercus acid," C, 50.2; H, 3.6. Titration equivalent about 400 (<sup>28</sup>,<sup>29</sup>).

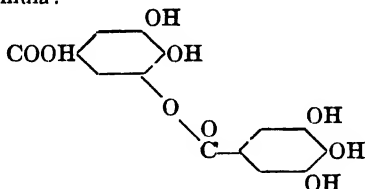
**Oak tannin, Quercitannic acid.**  $\gamma$ . B2. (1) Bark of various species of *Quercus* (<sup>28</sup>); Bark of *Quercus robur* (<sup>23</sup>). (2) Reddish-white powder (<sup>28</sup>). Light

- brown powder <sup>(23)</sup>. (3)  $C_{20}H_{20}O_8$  (?) : C, 59.79; H, 5.0 <sup>(24)</sup>. C, 56.8; H, 4.4. C, 55.4; H, 4.1 <sup>(25)</sup>. (6) Green <sup>(26)</sup>. Black-blue <sup>(27)</sup>.
- Oak tannin, Quercin, Quercic acid, Quercinic acid.**  $\gamma$ . B2. (1) Wood of various species of *Quercus*. (2) Light brownish-yellow <sup>(28)</sup>. (3)  $C_{14}H_{14}O_8 \cdot 2H_2O$  <sup>(29, 30)</sup>. C, 48.3; H, 4.5 <sup>(31)</sup>. (6) Blue.
- Paullinio tannin, Guarana tannin.**  $\gamma$ . B2. (1) "Guarana paste" from seeds of *Paullinia cupana*. (2) Small colorless crystals <sup>(32)</sup>. Gray needles <sup>(33)</sup>. (3)  $C_{27}H_{32}O_{13} \cdot COOH \cdot 2H_2O$  <sup>(34)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$ , ethyl acetate, glacial acetic acid <sup>(35)</sup>. (5)  $\alpha_D^{20}$ ,  $-74.4^\circ$  ( $H_2O$ , 10%);  $\alpha_D^{20}$ ,  $-39.1^\circ$  ( $C_2H_5OH$ , 8%);  $-48.1^\circ$  (acetone, 6%);  $\alpha_D^{20}$ ,  $-56.8^\circ$  (initial rotation in pyridine, 8%. By mutarotation falls to constant value of  $-8.6^\circ$ ) <sup>(36)</sup>. (7) M. P.  $199^\circ-201^\circ$  with evolution of  $CO_2$ . Loses two mol.  $H_2O$  of crystn. at  $130^\circ$ . M. P. of anhydrous form  $259^\circ-261^\circ$  with evolution of  $CO_2$  <sup>(37)</sup>. Paullinia catechol isolated from paullinia tannin is identical with "acacatechin" in crystal form and chemical properties. Chemically it is identical with gambier-catechin <sup>(38)</sup>.
- Pinicortannic acid.**  $\gamma$ . (1) Bark of Scotch fir, *Pinus sylvestris*. (2) Reddish-brown powder. (3)  $C_{15}H_{16}O_{11} \cdot H_2O$ . (6) Green <sup>(39)</sup>.
- Pistachio tannin.**  $\gamma$ . B2. (1) Leaves of mastic tree, *Pistachia lentiscus*. (2) Pale brown brittle mass <sup>(40)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$ , ethyl acetate <sup>(41)</sup>. (6) Blue-black. (7) Often sold for sumach <sup>(42)</sup>.
- Pomegranate tannin, Ellagitannin.**  $\beta$ . A3. (1) Root bark of *Punica granatum*. (2) Amorphous greenish-yellow powder. (3)  $C_{20}H_{10}O_{13}$  <sup>(43)</sup>. Two fractions: A (sol. in  $H_2O$ ), C, 50.9; H, 3.4. B (insol. in  $H_2O$ ), C, 52.4; H, 3.4 <sup>(44)</sup>. (4) Fraction A:  $H_2O$ ,  $C_2H_5OH$ , ethyl acetate <sup>(45)</sup>. (6) Blue-black. (7) Glucoside of ellagic acid and hexose <sup>(46)</sup>.
- Quebracho tannin.**  $\gamma$ . B2. (1) Wood of *Quebracho colorado*, *Schinopsis lorentzii* and *Balsanae*. (2) Red powder. (3) C, 62.5; H, 5.4. (4) Hot  $H_2O$ ,  $C_2H_5OH$ , ethyl acetate, acetone <sup>(47)</sup>. (6) Green. (7) Tannin is mixture of products insol. in  $H_2O$  and sparingly sol. in cold  $H_2O$ . A benzoyl derivative, C, 73.0; H, 4.2, showed a mol. wt. in benzene of about 2300.
- Rhatany tannin.**  $\gamma$ . Bark of root of rhatany, *Krameria triandra*. (2) Light yellow powder. (4)  $H_2O$ . (6) Green <sup>(48)</sup>.
- Rheotannic acid, Rhubarb tannin.**  $\gamma$ . B2. (1) Rhubarb. (2) Yellowish-brown powder. (3)  $C_{20}H_{16}O_{14}$  <sup>(49)</sup>. (4)  $H_2O$ . (6) Black-green ppt. (7) Contains two glucosides, glucogallin ( $C_{22}H_{18}O_{16}$ ) and tetrarin ( $C_{22}H_{18}O_{16}$ ) <sup>(50)</sup>. Catechin also present which is probably identical with gambier-catechin <sup>(51)</sup>.
- Rubitanic acid.**  $\gamma$ . (1) Leaves of *Rubia tinctorum*. (3)  $C_{14}H_{12}O_{13} \cdot 0.5H_2O$ . (6) Green <sup>(52)</sup>.
- Sequoiatannic acid.**  $\gamma$ . (1) Cones of *Sequoia gigantea*. (2) Reddish-brown powder. (3)  $C_{21}H_{20}O_{13}$  <sup>(53, 54)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$ . (6) Brown-black ppt.
- Spruce bark tannin.**  $\gamma$ . (1) Bark of spruce (3)  $C_{22}H_{18}O_{13}$  (?) <sup>(55)</sup>.
- Sumach tannin.**  $\alpha$ . A2. (1) From leaves of many species of *Rhus*. Also *Coriaria myrtifolia* (French), *Colpoon compressum* (Cape), *Arctostaphylos* (Russian). (2) Yellow powder. (3) C, 52.3; H, 3.5. (*Rhus coriaria*) <sup>(56)</sup>. (4)  $H_2O$ ,  $C_2H_5OH$ , ethyl acetate. (7) Similar to Turkish tannin.
- Tannecortepinic acid.**  $\gamma$ . (1) Bark of young Scotch firs in spring time. (3)  $C_{20}H_{18}O_{13}$ . (6) Green <sup>(57)</sup>.
- Tannic acid.** See Gallotannin.
- Tea tannin.**  $\gamma$ . A2. (1) Leaves of black tea. (4)  $H_2O$ , ethyl acetate. (5)  $\alpha_D^{20}$ ,  $-177.3^\circ$  <sup>(58)</sup>. (7) Probably identical with quercitannic acid <sup>(59, 60)</sup>. A gallotannin <sup>(61)</sup>.
- Tormentilla tannin.**  $\gamma$ . (1) Root of *Potentilla tormentilla*. (2) Amorphous reddish powder. (3)  $C_{20}H_{18}O_{11}$ . (6) Blue-green <sup>(62)</sup>.
- Turkish tannin.** See Gallotannin.
- Willow bark tannin.**  $\gamma$ . (1) Bark of *Salix triandra*. (6) Green. (7) Glucoside tannin <sup>(63)</sup>.

## TABLE XLV.

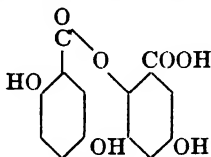
## CLASSIFICATION OF THE SYNTHESIZED TANNINS.

**m-Digallic acid. α. A1.** (2) Fine needles. (3) See below (<sup>12</sup>). (4)  $\text{CH}_3\text{OH}$ ,  $\text{C}_2\text{H}_5\text{OH}$ ,  $\text{C}_6\text{H}_{11}\text{OH}$ . 23°, 1 part in 950 parts  $\text{H}_2\text{O}$ ; 1 part in 350 parts ethyl acetate; 1 part in 2000 parts ether (<sup>4,5</sup>). 25°, 1 part in 1900 parts  $\text{H}_2\text{O}$ . 100°, 1 part in 50-60 parts  $\text{H}_2\text{O}$  (<sup>13</sup>). (5) Inactive. (6) Blue-black. (7) Found esterified with glucose in Chinese tannin. When hot aq. solution is chilled, it jellifies (<sup>12</sup>). M. P., 275° (282° corr.) with foaming and decomposition (<sup>4,5</sup>). Formula:



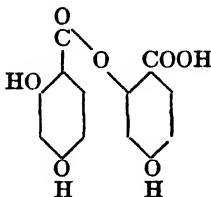
**Digalloyl-levoglucosan. A2.** (2) Micro-needles. (3)  $\text{C}_{20}\text{H}_{18}\text{O}_{13}$ . (4)  $\text{H}_2\text{O}$ ,  $\text{C}_2\text{H}_5\text{OH}$ , acetone. (5)  $\alpha_D^{25}$ , -27.9° ( $\text{C}_2\text{H}_5\text{OH}$ , 1.8%). (6)  $\text{FeCl}_3$  gives blue-black ppt. in  $\text{C}_2\text{H}_5\text{OH}$  solution. (7) Decomposes 220°, carbonizes 270° (<sup>14</sup>).

**Digentisic acid. α.** (2) Fine needles. (3) See below. (4) 0°, 1 part in 900 parts  $\text{H}_2\text{O}$ . (5) Inactive. (6) Fugitive blue and ppt. (<sup>4,5</sup>). (7) Dry form melts 204°-205° (208°-209° corr.) with sintering. Formula

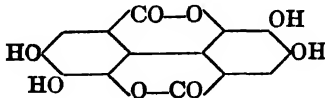


**Diprotocatechuic acid. α.** (2) Fine needles. (3)  $(\text{OH})_2\text{C}_6\text{H}_3\text{CO.O.C}_6\text{H}_3(\text{OH})\text{COOH}$ . (4) Acetone,  $\text{CH}_3\text{OH}$ . 1 part in 2500 parts  $\text{H}_2\text{O}$ . (5) Inactive. (6) Blue-green. (7) M. P., 237°-239° (corr.) (<sup>4,5</sup>).

**Di-β-resorcylic acid. α.** (2) Micro-needles. (3) See below. Isomeric with digentisic acid. (4)  $\text{C}_2\text{H}_5\text{OH}$ , acetone, ethyl acetate, hot  $\text{H}_2\text{O}$ , ether. (5) Inactive. (6) Violet red. (7) Fooms and decomposes at about 210° (215° corr.) (<sup>4,5</sup>). Formula:



**Ellagic acid. β.** (2) Cryst. from pyridine in prismatic needles which are converted by  $\text{C}_2\text{H}_5\text{OH}$  to a pale yellow cryst. powder. (3)  $\text{C}_{10}\text{H}_6\text{O}_8 \cdot 2\text{H}_2\text{O}$ . (5) inactive. (7) Above 360° sublims with carbonization (<sup>15</sup>). Not a true tannin. See Table XLIV. Formula:





- Hexagalloyl mannite. A2.** (2) Amorphous brown powder. (3)  $C_6H_5O_4$   $[CO_2C_6H_4(OH)]_6$ . (4)  $H_2O$ ,  $C_2H_5OH$ , acetone, ethyl acetate. (5)  $\alpha^*$   $+27.0^\circ$  ( $C_2H_5OH$ , 2%). (6) Dark blue ( $^{\circ}$ ).
- Maclurin. B1.** See Table XLIV.
- Penta-m-digalloyl- $\alpha$ -glucose. A2.** (2) Light brown amorphous mass. (3)  $C_{70}H_{122}O_{46}$ . (4)  $18^\circ$ , 1 part in 200 parts  $H_2O$ . (5) Prepared by alkaline hydrolysis of acetates:  $\alpha_D^{18}$   $+36^\circ$  ( $C_2H_5OH$ , 10%);  $+40^\circ$  to  $41^\circ$  (acetone, 10%);  $43.8^\circ$  ( $H_2O$ , 1%) ( $^{\circ}$ ). Prepared from acetates by  $CH_3OH$  and  $HCl$ :  $\alpha_D^{18}$   $+41.3^\circ$  ( $C_2H_5OH$ , 5%);  $+44.6^\circ$  (acetone, 5%);  $+51^\circ$  ( $H_2O$ , 0.5%) ( $^{\circ}$ ). (6) Blue-black. (7) Potassium salt containing 10.3% K,  $\alpha_D^{18}$   $+56.6^\circ$  ( $H_2O$ , 5%) ( $^{\circ}$ ).
- Penta-m-digalloyl- $\beta$ -glucose. A2.** (2) Light brown amorphous mass. (3)  $C_{70}H_{122}O_{46}$ . (4)  $20^\circ$ , 1 part in 1000 parts  $H_2O$ . (5) Prepared by alkaline hydrolysis of acetates:  $\alpha_D^{18}$   $+14.9^\circ$  ( $C_2H_5OH$ , 10%);  $+13.1^\circ$  (acetone, 10%);  $+42.3^\circ$  ( $H_2O$ , 1%) ( $^{\circ}$ ). Prepared from acetates by  $CH_3OH$  and  $HCl$ :  $\alpha_D^{18}$   $+10.8^\circ$  ( $C_2H_5OH$ , 5%);  $+10.8^\circ$  (acetone, 5%);  $+21^\circ$  ( $H_2O$ , 0.1%) ( $^{\circ}$ ). (6) Blue-black. (7) Apparently identical with Chinese tannin. Potassium salt containing 10.3% K,  $\alpha_D^{18}$   $+33.7^\circ$  ( $H_2O$ , 0.5%) ( $^{\circ}$ ).
- Pentagalloyl- $\alpha$ -glucose. A2.** (2) Yellow mass. (3)  $[(OH)_2C_6H_4CO]_5C_6H_4O_6$ . (4)  $H_2O$ ,  $C_2H_5OH$ , ether ( $^{\circ}$ ). (5)  $\alpha_D^{18}$   $+66.5^\circ$  ( $H_2O$ , 1%).  $\alpha_D^{23}$   $65.4^\circ$  ( $H_2O$ , 1%).  $\alpha_D^{30}$   $+77.0^\circ$  ( $C_2H_5OH$ , 3%).  $\alpha_D^{18}$   $76.4^\circ$  ( $C_2H_5OH$ , 2%) ( $^{\circ}$ ).  $\alpha_D^{18}$   $+60^\circ$  ( $H_2O$ , 1%);  $+81.5^\circ$  ( $C_2H_5OH$ , 2%) ( $^{\circ}$ ). (6) Blue-black.
- Pentagalloyl- $\beta$ -glucose. A2.** (2) Yellow mass. (3)  $[(OH)_2C_6H_4CO]_5C_6H_4O_6$ . (4)  $H_2O$ ,  $C_2H_5OH$  ( $^{\circ}$ ). (5)  $\alpha_D^{18}$   $+13.1^\circ$  ( $H_2O$ , 1%);  $+13.6^\circ$  ( $H_2O$ , 10%);  $+23.3^\circ$  ( $C_2H_5OH$ , 2%) ( $^{\circ}$ ).  $\alpha_D^{18}$   $+15^\circ$  ( $H_2O$ , 1%);  $+24^\circ$  ( $C_2H_5OH$ , 2%). (6) Blue-black. (7) Potassium salt contains 10.1% K.
- Pentapyrogallol-carboxyl-glucose. A2.** (2) Amorphous powder. (3)  $[(OH)_2C_6H_4CO]_5C_6H_4O_6$ . (4) Hot  $H_2O$ ,  $C_2H_5OH$ , acetone. (5)  $\alpha_D^{18}$   $+69^\circ$  ( $H_2O$ , 2.5%). (6) Dark blue ( $^{\circ}$ ). (7) Sinters at  $160^\circ$  and melts at about  $200^\circ$  with decomposition.
- Tetragalloyl-erythrite. A2.** (2) Cryst. (3)  $[(OH)_2C_6H_4CO]_4C_6H_4O_4$ . (4) Hot  $H_2O$ ,  $C_2H_5OH$ , acetone, mixtures of  $H_2O$  and  $C_2H_5OH$ . (7) Decomposes at about  $308^\circ$  ( $^{\circ}$ ).
- Tetragalloyl- $\alpha$ -methylglucoside. A2.** (3)  $C_{35}H_{50}O_{22}$ . (4) Identical with pentagalloyl-glucosides. (5)  $\alpha_D^{20}$   $+26.4^\circ$  ( $H_2O$ , 4%). (6) Identical with pentagalloyl-glucosides in reactions. (7) M. P.  $130^\circ$ – $140^\circ$  with decomposition ( $^{\circ}$ ).
- Trigalloyl-acetone-glucose. A2.** (2) Amorphous light brown mass. (3)  $[CaH_2(OH)_2CO]_3C_6H_4O_6(C_2H_5)_2$ . (4) Warm  $H_2O$ ,  $CH_3OH$ ,  $C_2H_5OH$ , acetone, ethyl acetate. (5)  $\alpha_D^{20}$   $-93^\circ$  (dry acetone, 4%). (6) Blue-violet ( $^{\circ}$ ).
- Trigalloyl-glucose. A2.** (2) Amorphous yellowish brown mass. (3)  $[CaH_2(OH)_2CO]_3C_6H_4O_6$ . (4) Cold  $H_2O$ ,  $CH_3OH$ ,  $C_2H_5OH$ , acetone, ethyl acetate, pyridine. (5)  $\alpha_D^{20}$   $-118^\circ$  (dry acetone, 2.5%). (6) Deep violet ( $^{\circ}$ ).
- Trigalloyl-glycerol. A2.** (2) Amorphous yellowish brown mass. (3)  $[(OH)_2C_6H_4CO]_3C_3H_5O_3$ . (4)  $H_2O$ , acetone, ethyl acetate, warm ether. (6) Deep blue ( $^{\circ}$ ).
- $\alpha$ -Trigalloyl-levoglucosan. A2.** (2) Micro. hexagonal crystals. (3)  $C_{27}H_{20}O_{11}$ . (4) Hot acetone. (5)  $\alpha_D^{18}$   $-18.0^\circ$  ( $C_2H_5OH$ , 19%). (6)  $FeCl_3$  gives blue-black ppt. in  $C_2H_5OH$  solution. (7) Decomposes  $250^\circ$ – $300^\circ$ , carbonizes  $320^\circ$  ( $^{20}$ ).
- $\beta$ -Trigalloyl-levoglucosan. A2.** (2) Micro-needles. (3)  $C_{27}H_{20}O_{11}$ . (5)  $\alpha_D^{18}$   $-21.0^\circ$  ( $C_2H_5OH$ , 1%). (6)  $FeCl_3$  gives blue-violet ppt. in  $C_2H_5OH$  solution. (7) Decomposes  $270^\circ$ , carbonizes  $320^\circ$  ( $^{20}$ ).

## TABLE XLVI

## CLASSIFICATION OF THE CATECHOLS OR CATECHINS

- d-Catechol.** (1) Acacia and gambier catechus. (2) Thin needles. (3)  $C_{15}H_{14}O_6 \cdot 4H_2O$  ( $^{20}$ ). (4)  $C_2H_5OH$ , ethyl acetate, pure ether. Anhydrous form almost insoluble in latter two. (5)  $\alpha_{D^{18}}$   $+17^\circ$  (50% acetone, 9%) ( $^{14, 15, 16, 20}$ ).  $\alpha_{D^{18}}$   $\pm 0^\circ$  ( $C_2H_5OH$  ( $^{20}$ )).  $\alpha_D$   $-2^\circ$  ( $C_2H_5OH$ ) ( $^{20}$ ).  $\alpha_D^{18}$   $-0.47^\circ \pm 0.03^\circ$

( $\text{C}_2\text{H}_5\text{OH}$ , 9%);  $+3.7^\circ \pm 0.5^\circ$  (50%  $\text{C}_2\text{H}_5\text{OH}$ , 9%).  $\alpha_D^{20}$ ,  $+18.4^\circ \pm 0.9^\circ$  ( $\text{H}_2\text{O}$ , 0.9%, increasing markedly with temperature decrease) (<sup>14</sup>). (7) For discussion of structural formula see (<sup>20</sup>, <sup>22</sup>, <sup>27</sup>). M. P.  $93^\circ\text{--}95^\circ$ ; anhyd.,  $174\text{--}5^\circ$ .

***l*-Catechol.** (1) Isolated from acacia and gambier catechus. (2) Thin needles. (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6 \cdot 4\text{H}_2\text{O}$ . (5)  $\alpha_{\text{D}}^{25}$ ,  $\pm 0^\circ$  ( $\text{C}_2\text{H}_5\text{OH}$ );  $\alpha_{\text{D}}^{25}$ ,  $-16.8^\circ$  (50% acetone, 3%) (<sup>20</sup>). (7) M. P.  $93^\circ\text{--}95^\circ$ ; anhyd.,  $174^\circ\text{--}175^\circ$  (<sup>19</sup>, <sup>20</sup>).

***dl*-Catechol.** (1) Principal constituent of catechol separated from acacia catechu. (2) Thin needles. (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6 \cdot 3\text{H}_2\text{O}$ . (7) Is "acacatechin" (<sup>19</sup>). Sinters at  $100^\circ$ , melts  $214^\circ\text{--}216^\circ$  with decomposition (<sup>19</sup>, <sup>20</sup>).

**Catechol-a.** (1) Acacia catechu (<sup>21</sup>, <sup>22</sup>). (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6 \cdot 3\text{H}_2\text{O}$  (<sup>20</sup>). (7) Is *dl*-catechol (<sup>14</sup>). Methylated "acacatechin" has same melting point and crystal form as synthetic methyl compound (<sup>14</sup>). M. P.  $204^\circ\text{--}205^\circ$  (<sup>20</sup>).

**Catechol-b, Gambier catechol.** (1) Gambier catechu (<sup>21</sup>, <sup>22</sup>). (7) Identical with *d*-catechol in crystal form, melting point, solubility, and constitution.

**Catechol-c.** (1) Gambier catechu. (2) Small pale yellow prisms (<sup>20</sup>). (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6$ . (7) Identified as *d*-epicatechol (<sup>20</sup>). M. P.  $235^\circ\text{--}237^\circ$  (<sup>20</sup>).

**Chinese rhubarb catechol.** (5)  $\alpha_{\text{D}}^{25}$ ,  $+18^\circ$  (50% acetone) (<sup>15</sup>).

**Mahogany catechol.** (5)  $\alpha_D$ ,  $+23^\circ$  (50% acetone).  $\alpha_{\text{D}}^{25}$ ,  $+16^\circ$  (50% acetone);  $+15^\circ$  ( $\text{C}_2\text{H}_5\text{OH}$ ) (<sup>15</sup>).

**Paulinia catechol.** (5) Inactive in  $\text{C}_2\text{H}_5\text{OH}$ .  $\alpha_D$ ,  $+3.7^\circ$  (50% acetone) (<sup>15</sup>).

***d*-Epicatechol.** (2) Thick prisms. (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6 \cdot 4\text{H}_2\text{O}$ . (5)  $\alpha_{\text{D}}^{25}$ ,  $+68.9^\circ$  ( $\text{C}_2\text{H}_5\text{OH}$ , 7%);  $+59.9^\circ$  (50% acetone, 4%). (7) M. P.  $245^\circ$  (<sup>20</sup>).

***l*-Epicatechol.** (1) Gambier and acacia catechus (<sup>19</sup>). (2) Thick prisms. (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6 \cdot 4\text{H}_2\text{O}$  (<sup>19</sup>, <sup>20</sup>). (5)  $\alpha_{\text{D}}^{25}$ ,  $-68.2^\circ$  ( $\text{C}_2\text{H}_5\text{OH}$ , 6%);  $-59.0^\circ$  (50% acetone, 4%) (<sup>19</sup>, <sup>20</sup>). (7) M. P.  $245^\circ$ .

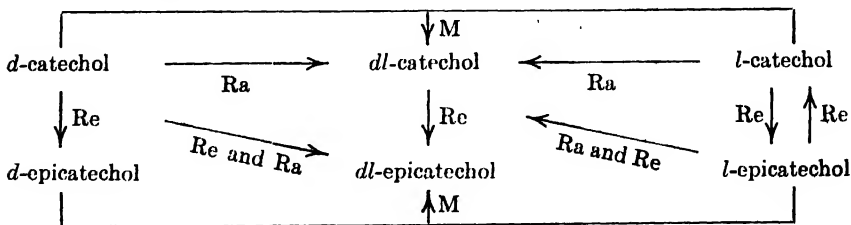
***dl*-Epicatechol.** (1) Gambier and acacia catechus (<sup>19</sup>). (2) Exists both as prisms and needles. (3)  $\text{C}_{12}\text{H}_{10}\text{O}_6 \cdot 4\text{H}_2\text{O}$ . (7) M. P. of prisms,  $224^\circ\text{--}226^\circ$  (<sup>20</sup>).

***d*- $\beta$ -Gambier catechol-carboxylic acid.** (2) Micro-needles. (3)  $\text{C}_{16}\text{H}_{14}\text{O}_8$ . (5)  $\alpha_D^{20}$ ,  $+12.6^\circ$  ( $\text{H}_2\text{O}$ , 5%);  $+17.6^\circ$  ( $\text{C}_2\text{H}_5\text{OH}$ , 7%). (7) M. P.  $249^\circ\text{--}251^\circ$  with evolution of  $\text{CO}_2$  (<sup>22</sup>).

***l*- $\beta$ -Gambier catechol-carboxylic acid.** (2) Large needles. (3)  $\text{C}_{16}\text{H}_{14}\text{O}_8$ . (5)  $\alpha_D^{18}$ ,  $-22.4^\circ$  ( $\text{H}_2\text{O}$ , 5%).  $\alpha_D^{17}$ ,  $-31.6^\circ$  ( $\text{C}_2\text{H}_5\text{OH}$ , 6%). (7) M. P.  $258^\circ\text{--}261^\circ$  with evolution of  $\text{CO}_2$  (<sup>22</sup>).

***dl*- $\beta$ -Gambier catechol-carboxylic acid.** (3)  $\text{C}_{16}\text{H}_{14}\text{O}_8$ . (7) M. P.  $252^\circ\text{--}253^\circ$  with evolution of  $\text{CO}_2$  (<sup>22</sup>).

The relationship between the catechols and epicatechols is shown as follows:



### Physical Chemistry of the Tannins.

Although very much more work has been done upon the organic chemistry of the tannins than upon the physical chemistry, the latter has proved much more effective in the development of theories of the mechanism of tanning. This may be attributed to the fact that the organic chemistry of the proteins, tannins, and nontannins is still far too complex to permit satisfactory reasoning regarding their chemical

reactions with one another. On the other hand, a number of relatively simple principles of physical chemistry seem to apply to the tanning reactions. These will be discussed in connection with the Procter-Wilson theory of tanning in the chapter on Vegetable Tanning in Volume Two.

### Potential Difference of Tannin Solutions.

In Chapter 5, it was pointed out that the stability of a colloidal dispersion is determined less by the absolute value of the electrical charge on the particles than by the electrical difference of potential between the film of solution wetting the particles and the bulk of the surrounding solution. In the Procter-Wilson theory, the astringency of a tan liquor in practice is assumed to be a function of the potential difference between the solution immediately in contact with the tannin particles and the bulk of the tan liquor as well as of the potential difference between the tan liquor and the collagen jelly. Grasser<sup>27</sup> studied the electro-chemistry of tannin solutions, but obtained confusing results of rather doubtful value, which may be due to his failure to control or measure the hydrogen-ion concentrations of the liquors.

Thomas and Foster<sup>43</sup> were more successful. Using the U-tube electrophoresis method described by Burton,<sup>1</sup> they succeeded in measuring the potential differences of tannin solutions under different conditions. Table XLVII shows a series of values obtained for tan liquors made from 8 typical tanning materials. It is interesting to find gambier, the mildest tanning material, with the lowest potential difference and quebracho, the most astringent, with the highest potential difference. The order of decreasing conductivity of these solutions was sumac, gambier, oak bark, larch bark, hemlock bark, chestnut wood, osage orange, quebracho. It is evident that the potential difference is not a simple function of the conductivity, but is influenced by the kind as well as the amount of electrolyte present.

TABLE XLVII

POTENTIAL DIFFERENCES OF TANNINS FROM DIFFERENT SOURCES.

Extract	Grams Total Soluble Matter Per Liter	Potential Difference Volts
Gambier (cube) .....	18.7	—0.005
Oak bark .....	17.0	—0.009
Chestnut wood .....	17.8	—0.009
Hemlock bark .....	16.7	—0.010
Sumac .....	19.6	—0.014
Larch bark .....	19.5	—0.018
Osage orange .....	13.7	—0.018(?)
Quebracho .....	11.0	—0.028

If the absolute value of the electrical charge on the particles remains constant, according to the theory given in Chapter 5, the potential difference at the surface should decrease with increasing concentration of electrolyte, or increase with decreasing concentration. Thomas and Foster found that the potential difference of solutions of quebracho extract actually does increase with decreasing concentrations, as shown in Table XLVIII. The addition of acid decreases the value of the potential difference by lowering the absolute value of the electrical charge, which holds true for negatively charged dispersions in general. This is shown in Table XLIX.

TABLE XLVIII  
POTENTIAL DIFFERENCES OF SOLUTIONS OF QUEBRACHO EXTRACT.

Concentration Grams Dry Solids Per Liter	Potential Difference Volts
32	— 0.024
16	— 0.028
8	— 0.029
4	— 0.030

The effect of dialyzing a tan liquor is to lower the concentration of electrolyte, which we should expect to increase the potential difference. The values in Table L show that this actually occurs, although part of the increase may be attributed to dilution.

TABLE XLIX  
EFFECT OF ADDITION OF ACID.

(16 grams of solid quebracho extract per liter.)	
0.1N HCl Added Per Liter Cubic Centimeters	Potential Difference Volts
0	— 0.024
10	— 0.014
15	— 0.010
20	approx. 0

TABLE L  
EFFECT OF DIALYSIS.

Extract	Grams Extract in 250 cc.	Hours Dialyzed	Final Volume cc.	Potential Difference Volts
Quebracho .....	4	60	415	— 0.033
Osage orange .....	4	24	370	— 0.024
Sumac .....	4	24	460	— 0.026
Gambier .....	8.2	24	390	— 0.029
Hemlock bark .....	...	24	...	— 0.024

### Isoelectric Points of the Tannins.

Thomas and Foster<sup>44</sup> later extended their investigations in an attempt to determine the isoelectric points of tannins from different sources. The various tanning extracts were dissolved in a citrate buffer mixture having a pH value of 2.0 and the solutions were finally adjusted to the desired pH values by means of the hydrogen electrode. The buffer was apparently necessary to eliminate, or delay, the secondary actions, such as diffusion of the boundaries and change of reaction of the extracts due to electrolysis, which behavior had nullified previous experiments.

Between the pH values 2.5 and 2.0, the direction of migration of the tannin particles changed from anodic to cathodic in solutions of the extracts of oak bark, hemlock bark, wattle bark, sumac, and gambier. In the case of quebracho, there seemed to be no movement in the U-tube at the pH values 3.0 or 2.5, but at 2.0 the movement seemed to be slightly cathodic. Quebracho was precipitated by the buffer and only the clear, supernatant liquor could be used, which may account for the inability to obtain more definite results.

Until they are located more definitely, the isoelectric points of the tannins may be accepted as lying between the pH values 2.0 and 2.5, at least those of hemlock, oak, and wattle barks, sumac, and gambier.

The isoelectric points of the proteins are generally at pH values much higher than 2.5, as, for example, the isoelectric point of gelatin at 4.7. Proteins are amphoteric, dissociating as both acid and base, and the isoelectric point occurs where the acidic and basic dissociations are equal. We have, as yet, no reason to look upon tannins as being amphoteric. With increasing concentration of hydrogen ion, there is apparently an accumulation of hydrogen ion at the surface of the particles, whose positive charge tends to neutralize the negative charge due to the acidic dissociation of the tannins. At pH values below 2.0, the accumulation of hydrogen has more than offset the slight acidic dissociation and the particles become positively charged.

### Precipitation of Tan Liquors.

In the hope of throwing some light upon the colloidal nature of the tannins, Thomas and Foster studied the action of various electrolytes upon a great variety of tan liquors. Aqueous solutions of different tanning extracts were made up so that 100 cubic centimeters of solu-

tion contained 4 grams of solid matter. The solutions were made at 85° C., cooled to 25°, and then adjusted to final volume. The stock solution was then centrifuged for 5 minutes at 1000 times gravity in order to throw down coarse suspended matter. Portions of 25 cubic centimeters were put into 100-cubic centimeter, graduated oil tubes. Then 25 cubic centimeters of the electrolyte were added, the solutions were allowed to stand for 15 to 30 minutes for precipitation to start, and were then centrifuged for 5 minutes at 1000 times gravity. The volumes of the precipitates were recorded and plotted against the concentrations of electrolyte employed.

The results may be most conveniently studied by grouping them under the names of the various electrolytes used. Each available extract was not tested with all electrolytes because, in some cases, preliminary experiments indicated that further work would be fruitless.

### Monovalent Cations.

**Potassium chloride.** Concentrations of potassium chloride from 0.02 to 4 molar gave only negligible amounts of precipitate with gambier and quebracho. Oak bark gave a gradually increasing salting

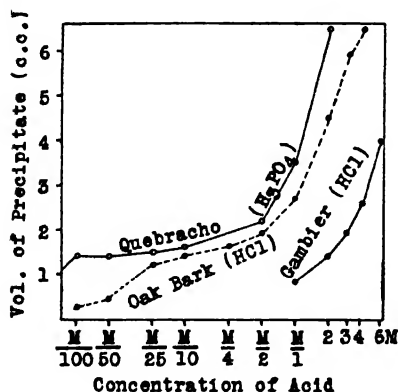


FIG. 95.—Precipitation of Tannins by Hydrochloric and Phosphoric Acids.

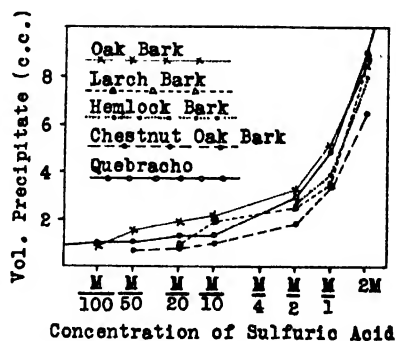


FIG. 96.—Precipitation of Tannins by Sulfuric Acid.

out effect. Since gambier and quebracho represent extreme types of tanning extracts, no further tests were made with this salt. It must be borne in mind that the solutions to which the neutral salts were added were made simply by dissolving the extracts in distilled water and had pH values in the vicinity of 4.5.

**Hydrochloric acid.** Concentrations from 0.01 to 6 molar were used. Gambier and quebracho gave large amounts of precipitate only at high concentrations of acid and, since this was not a simple colloid precipitation, no further experiments were attempted. A salting out effect was obtained with oak bark. (See Fig. 95.)

**Sulfuric acid.** Quebracho, hemlock bark, oak bark, and larch bark gave progressively increasing amounts of precipitate with increasing concentration of acid, as shown in Fig. 96. No precipitate was obtained with sumac until molar concentration was reached, when gummy masses were thrown down, similar to those obtained with aluminum sulfate. At 4-molar concentration, a flocculent precipitate was formed.

**Phosphoric acid.** Gambier began to give an appreciable precipitate only at 4 to 7-molar concentration. With sumac a gummy mass was thrown out at 2-molar, as was observed upon the addition of sulfuric acid and aluminum sulfate, and at 4 to 7-molar a flocculent precipitate formed which left the supernatant solution almost colorless. Quebracho was progressively salted out. (See Fig. 95.)

**Acetic acid.** Experiments with quebracho, sumac, gambier, and oak bark were run with concentrations of acid from 0.005 to 4 molar. There was no appreciable precipitation in any case. At the higher concentrations the suspended matter began to dissolve.

**Formic acid.** Concentrations from 0.005 to 12.5 molar were used. Sumac, chestnut oak bark, larch bark, gambier, and hemlock bark

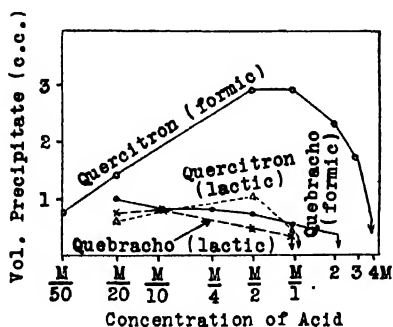


FIG. 97.—Precipitation of Tannins by Formic and Lactic Acids.

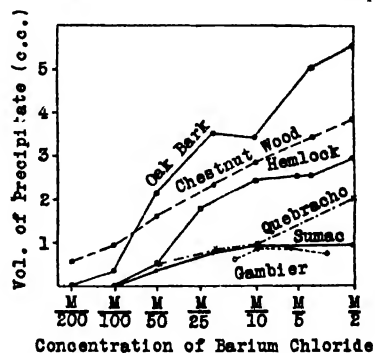


FIG. 98.—Precipitation of Tannins by Barium Chloride.

gave no precipitation up to 4 molar, at which concentration the suspended matter began to dissolve. Quebracho and quercitron bark were precipitated, but the precipitate redissolved at from 2 to 4 molar. (See Fig. 97.)

**Lactic acid.** Concentrations from 0.005 to 2 molar were employed. The effects of this acid were similar in kind, but not in degree, to those with formic acid. (See Fig. 97.) The precipitates with quebracho and quercitron redissolved at lower concentrations of lactic than of formic acid. Since lactic is the weaker acid and since this redissolving was not found with hydrochloric or sulfuric acids, the effect must be due to chemical properties other than those of the hydrogen ion. This is an important point to consider in the chemical control of tan liquors.

### Divalent Cations.

**Barium chloride.** On account of the limit of solubility, this salt was employed up to only 0.5 molar. The salting out effect is shown in Fig. 98.

**Calcium chloride.** Concentrations up to 2 molar were used. As with barium chloride, increasing amounts of precipitate were obtained with the different tanning materials used, as shown in Fig. 99. At the same concentration of these salts the different extracts gave in some

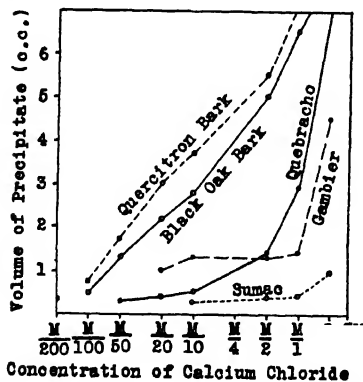


FIG. 99.—Precipitation of Tannins by Calcium Chloride.

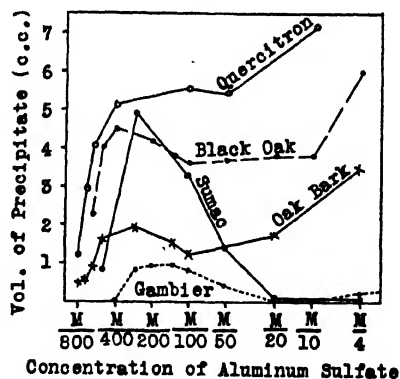


FIG. 100.—Precipitation of Tannins by Aluminum Sulfate.

cases less, and in others more, precipitate, showing the presence of substances reacting with barium and calcium ions to form compounds of different solubilities.

### Trivalent Cation.

**Aluminum sulfate.** In the precipitation of negatively charged colloidal dispersions, aluminum sulfate is not only a powerful precipitant, but it also gives the "irregular series" or "tolerance zone" which is typical of the action of weak base cation-strong acid anion



salts, as shown by Buxton and Teague,<sup>2</sup> and by Freundlich and Schucht.<sup>26</sup> The concentrations of aluminum sulfate used ranged from 0.00125 to 0.5 molar.

The "irregular series" effect was obtained with gambier, sumac,

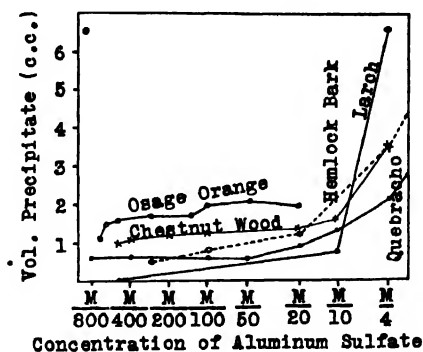


FIG. 101.—Precipitation of Tannins by Aluminum Sulfate.

oak bark, and quercitron bark. Precipitation generally set in at 0.00125 molar concentration, rose rapidly to a maximum, dropped off

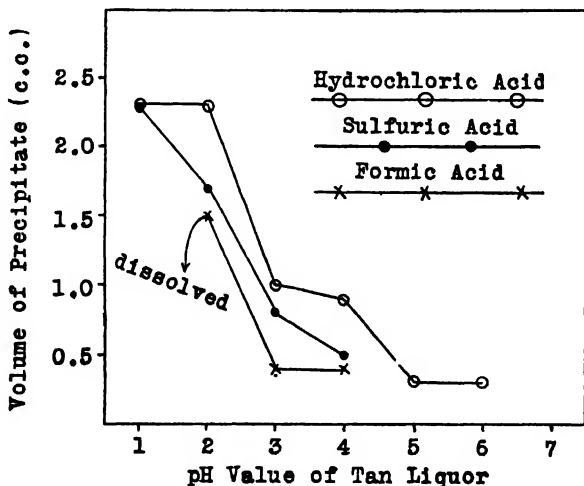


FIG. 102.—Precipitation of Tannins of Quebracho Extract as a Function of pH Value.

into a "tolerance zone," and then started upward again, as shown in Fig. 100.

Those which gave no "irregular series," at least up to 0.5 molar concentration of the salt, were osage orange, quebracho, camel cutch,

chestnut wood, chestnut oak bark, hemlock bark, and larch bark, shown in Fig. 101. Precipitation started at 0.00125 molar and increased gradually to about 0.1 molar, where there was an abrupt upward trend similar to a salting out effect. These extracts are not so sensitive to precipitation by dilute solutions of aluminum sulfate as those shown

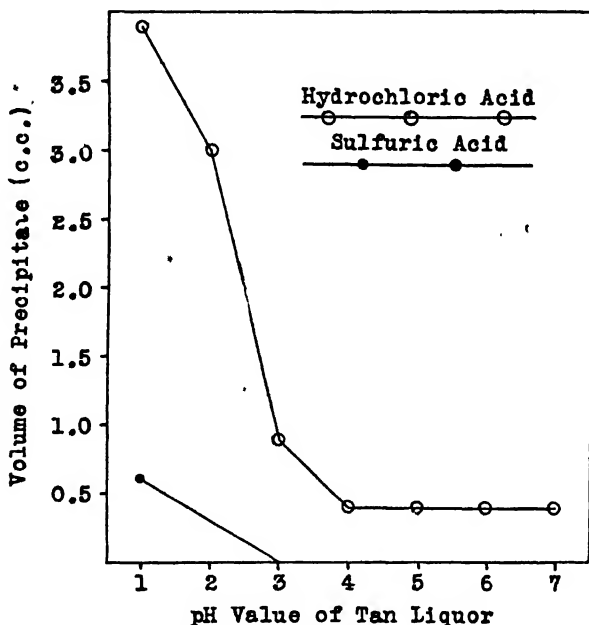


FIG. 103.—Precipitation of Tannins of Gambier Extract as a Function of pH Value.

in Fig. 100. Bengal cutch seemed to be in a separate category, since it was unaffected by the addition of aluminum sulfate.

#### Hydrogen-Ion Concentration.

The effect of hydrogen-ion concentration upon the precipitation of solutions of quebracho, gambier, larch bark, and oak bark by sulfuric, hydrochloric, and formic acids is shown in Figs. 102, 103, 104, and 105. Solutions of sumac, hemlock bark, and wattle bark were not precipitated by these acids with increasing acidity to  $\text{pH} = 1$ . It is evident that the volume of precipitate formed is not a function of hydrogen-ion concentration alone, since the three acids give curves of different shapes.

Wherever a precipitate formed, the amount invariably increased

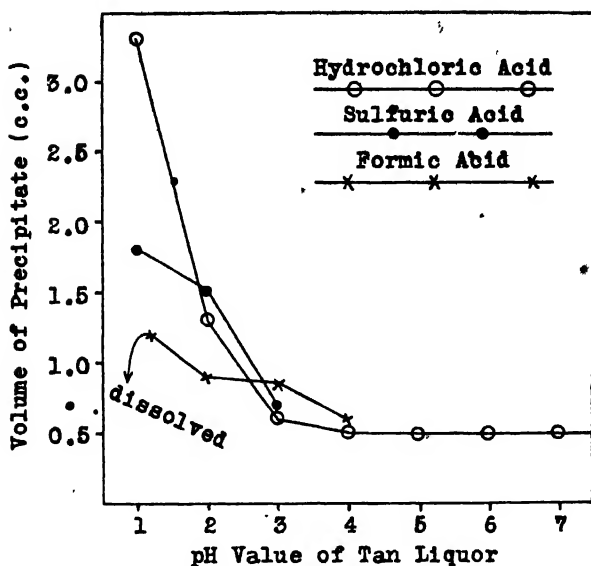


FIG. 104.—Precipitation of Tannins of Larch Bark Extract as a Function of pH Value.

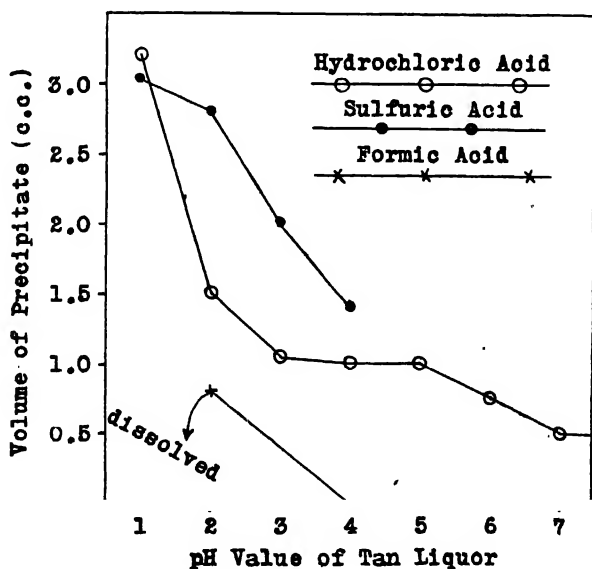


FIG. 105.—Precipitation of Tannins of Oak Bark Extract as a Function of pH Value.

with increasing hydrogen-ion concentration where hydrochloric and sulfuric acids were used. But an increasing concentration of formic acid dissolved the precipitate, or the suspended matter in cases where no precipitate had previously formed.

The precipitates obtained with hydrochloric acid were found to be soluble in strong alcohol and in 9-molar lactic acid. On shaking up with water, these precipitates dispersed, but gradually settled out more or less completely in 24 hours. In the case of oak bark and quebracho, it was found that approximately two-thirds of the original solid matter present had been precipitated at  $\text{pH} = 1$ .

When the pH value was increased by the addition of sodium hydroxide, there was increasing solution, clear liquids being obtained in every case at  $\text{pH} = 8$ . The effect of adding calcium hydroxide, however, is very different, as will be recalled from Fig. 86 of Chapter 12. At pH values above 7.2 increasing amounts of precipitate are obtained with increasing pH value.

Solutions of tanning extracts behave in many ways like colloidal dispersions with properties lying between those of the intermediate and hydrophilic systems and in other ways like true crystalloidal solutions of organic acids.

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